

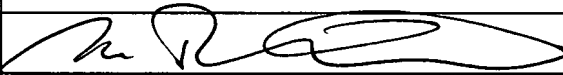
TRANSMITTAL FORM (To be used for all correspondence after initial filing)	Patent Number	6,309,650
	Issue Date	October 30, 2001
	First Named Inventor	Hyun Su Kim
	Art Unit	
	Examiner Name	
	Attorney Docket No.	201131.401USPØ

RECEIVED

JUN 02 2009

OFFICE OF PETITIONS

ENCLOSURES (check all that apply)		
<input checked="" type="checkbox"/> Fee Transmittal Form <input type="checkbox"/> Fee Attached <input type="checkbox"/> Amendment/Response <input type="checkbox"/> After Final <input type="checkbox"/> Affidavits/declaration(s) <input type="checkbox"/> Extension of Time Request <input type="checkbox"/> Express Abandonment Request <input type="checkbox"/> Information Disclosure Statement and Transmittal <input type="checkbox"/> Cited References <input type="checkbox"/> Certified Copy of Priority Document(s) <input type="checkbox"/> Response to Missing Parts under 37 CFR 1.52 or 1.53 <input type="checkbox"/> Response to Missing Parts/Incomplete Application	<input type="checkbox"/> Drawing(s) <input type="checkbox"/> Request for Corrected Filing Receipt <input type="checkbox"/> Licensing-related Papers <input type="checkbox"/> Petition <input type="checkbox"/> Petition to Convert to a Provisional Application <input type="checkbox"/> Power of Attorney, Revocation, Change of Correspondence Address <input type="checkbox"/> Declaration <input type="checkbox"/> Statement under 37 CFR 3.73(b) <input type="checkbox"/> Terminal Disclaimer <input type="checkbox"/> Request for Refund <input type="checkbox"/> CD, Number of CD(s) _____ <input type="checkbox"/> Landscape Table on CD	<input type="checkbox"/> After Allowance Communication to TC <input type="checkbox"/> Appeal Communication to Board of Appeals and Interferences <input type="checkbox"/> Appeal Communication to TC (<i>Appeal Notice, Brief, Reply Brief</i>) <input type="checkbox"/> Proprietary Information <input type="checkbox"/> Status Letter <input checked="" type="checkbox"/> Return Receipt Postcard <input checked="" type="checkbox"/> Other Enclosure(s) (please identify below): <u>Application for Extension of Patent Term;</u> <u>Exhibits A-M;</u> <u>Two Additional Copies</u>
Remarks		

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT			
Firm Name	Seed Intellectual Property Law Group PLLC		Customer Number 00500
Signature			
Printed Name	William T. Christiansen, Ph.D.		
Date	May 28, 2009	Reg. No.	44,614

CERTIFICATE OF TRANSMISSION/MAILING		
I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below.		
Signature		
Typed or printed name		Date: RECEIVED MAY 28 2009

SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.
1397200_1.DOC

PATENT EXTENSION
OPLA

Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL

For FY 2009

Complete if Known

Patent Number	6,309,650
Issue Date	October 30, 2001
First Named Inventor	Hyun Su Kim
Examiner Name	
Art Unit	
Attorney Docket No.	201131.401US

☐ Applicant claims small entity status. See 37 CFR 1.27**TOTAL AMOUNT OF PAYMENT** (\$1,120)**METHOD OF PAYMENT** (check all that apply)☐ Check ☐ Credit Card ☐ Money Order ☐ Other (please identify): _____☒ Deposit Account Deposit Account Number: 19-1090 Deposit Account Name: Seed IP Law Group PLLC

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

☒ Charge fee(s) indicated below☐ Charge fee(s) indicated below, **except for the filing fee**☒ Charge any additional fee(s) or underpayments☒ Charge any underpayments or credit any overpayments

of fee(s) under 37 CFR 1.16 and 1.17

Warning: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.**FEE CALCULATION****1. BASIC FILING, SEARCH, AND EXAMINATION FEES**

Application Type	FILING FEES		SEARCH FEES		EXAMINATION FEES		Fees Paid (\$)
	Fee (\$)	<u>Small Entity</u> Fee (\$)	Fee (\$)	<u>Small Entity</u> Fee (\$)	Fee (\$)	<u>Small Entity</u> Fee (\$)	
Utility	330	165	540	270	220	110	_____
Design	220	110	100	50	140	70	_____
Provisional	220	110	0	0	0	0	_____

2. EXCESS CLAIM FEES

Fee Description	Fee (\$)	<u>Small Entity</u> Fee (\$)
Each claim over 20 (including Reissues)	52	26
Each independent claim over 3 (including Reissues)	220	110
Multiple dependent claims	390	195

Total Claims	Extra Claims	Fee (\$)	Fee Paid (\$)	Multiple Dependent Claims	
_____ -20 or HP = _____	X	_____	_____	Fee (\$)	Fee Paid (\$)

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims	Extra Claims	Fee (\$)	Fee Paid (\$)
_____ -3 or HP = _____	X	_____	_____

HP = highest number of independent claims paid for, if greater than 3.

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is \$270 (\$135 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof	Fee (\$)	Fee Paid (\$)
_____ -100 = _____	/50 = _____	_____ (round up to a whole number)	x _____	_____

4. OTHER FEE(S)

Non-English Specification, \$130 fee (no small entity discount)

Other (e.g., late filing surcharge): Extension of Term Patent


RECEIVED

MAY 28 2009

PATENT EXTENSION
OPLA**Fees Paid (\$)**

1,120

SUBMITTED BY

Signature		Registration No. (Attorney/Agent)	44,614	Telephone	206-622-4900
Name (Print/Type)	William T. Christiansen, Ph.D.	Date	May 28, 2009		

Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL

For FY 2009

Complete if Known

Patent Number	6,309,650
Issue Date	October 30, 2001
First Named Inventor	Hyun Su Kim
Examiner Name	
Art Unit	
Attorney Docket No.	201131.401USPC

RECEIVED
JUN 2 2009

OFFICE OF PETITIONS

☐ Applicant claims small entity status. See 37 CFR 1.27TOTAL AMOUNT OF PAYMENT (\$)**1,120**

METHOD OF PAYMENT (check all that apply)

☐ Check ☐ Credit Card ☐ Money Order ☐ Other (please identify): _____☒ Deposit Account Deposit Account Number: 19-1090 Deposit Account Name: Seed IP Law Group PLLC

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

☒ Charge fee(s) indicated below ☐ Charge fee(s) indicated below, **except for the filing fee**
☒ Charge any additional fee(s) or underpayments ☒ Charge any underpayments or credit any overpayments of fee(s) under 37 CFR 1.16 and 1.17

Warning: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

Application Type	FILING FEES		SEARCH FEES		EXAMINATION FEES		Fees Paid (\$)
	Fee (\$)	<u>Small Entity</u> Fee (\$)	Fee (\$)	<u>Small Entity</u> Fee (\$)	Fee (\$)	<u>Small Entity</u> Fee (\$)	
Utility	330	165	540	270	220	110	_____
Design	220	110	100	50	140	70	_____
Provisional	220	110	0	0	0	0	_____

2. EXCESS CLAIM FEES

Fee Description	Fee (\$)	<u>Small Entity</u> Fee (\$)
Each claim over 20 (including Reissues)	52	26
Each independent claim over 3 (including Reissues)	220	110
Multiple dependent claims	390	195

Total Claims	Extra Claims	Fee (\$)	Fee Paid (\$)	Multiple Dependent Claims	
_____ -20 or HP = _____	X	_____	_____	Fee (\$)	Fee Paid (\$)

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims	Extra Claims	Fee (\$)	Fee Paid (\$)
_____ -3 or HP = _____	X	_____	_____

HP = highest number of independent claims paid for, if greater than 3.

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is \$270 (\$135 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof	Fee (\$)	Fee Paid (\$)
_____ -100 = _____	/50 = _____	_____ (round up to a whole number)	_____	_____

4. OTHER FEE(S)

Non-English Specification, \$130 fee (no small entity discount)


Other (e.g., late filing surcharge): Extension of Term Patent

RECEIVED
MAY 28 2009
PATENT EXTENSION
OPLA

Fees Paid (\$)

1,120

SUBMITTED BY

Signature		Registration No. (Attorney/Agent)	44,614	Telephone	206-622-4900
Name (Print/Type)	William T. Christiansen, Ph.D.			Date	May 28, 2009

Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL

For FY 2009

Complete if Known

Patent Number	6,309,650
Issue Date	October 30, 2001
First Named Inventor	Hyun Su Kim
Examiner Name	
Art Unit	
Attorney Docket No.	201131.401USPC

☐ Applicant claims small entity status. See 37 CFR 1.27**TOTAL AMOUNT OF PAYMENT** (\$)**1,120****METHOD OF PAYMENT (check all that apply)**☐ Check ☐ Credit Card ☐ Money Order ☐ Other (please identify): _____☒ Deposit Account Deposit Account Number: 19-1090 Deposit Account Name: Seed IP Law Group PLLC

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

☒ Charge fee(s) indicated below☐ Charge fee(s) indicated below, **except for the filing fee**☒ Charge any additional fee(s) or underpayments☒ Charge any underpayments or credit any overpayments

of fee(s) under 37 CFR 1.16 and 1.17

Warning: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.**FEE CALCULATION****1. BASIC FILING, SEARCH, AND EXAMINATION FEES**

Application Type	FILING FEES		SEARCH FEES		EXAMINATION FEES		Fees Paid (\$)
	Fee (\$)	<u>Small Entity</u> Fee (\$)	Fee (\$)	<u>Small Entity</u> Fee (\$)	Fee (\$)	<u>Small Entity</u> Fee (\$)	
Utility	330	165	540	270	220	110	_____
Design	220	110	100	50	140	70	_____
Provisional	220	110	0	0	0	0	_____

2. EXCESS CLAIM FEES**Fee Description**

Each claim over 20 (including Reissues)

Each independent claim over 3 (including Reissues)

Multiple dependent claims

<u>Total Claims</u>	<u>Extra Claims</u>	<u>Fee (\$)</u>	<u>Fee Paid (\$)</u>	<u>Multiple Dependent Claims</u>
_____ -20 or HP = _____	X	_____	_____	<u>Fee (\$)</u> <u>Fee Paid (\$)</u>

HP = highest number of total claims paid for, if greater than 20.

<u>Indep. Claims</u>	<u>Extra Claims</u>	<u>Fee (\$)</u>	<u>Fee Paid (\$)</u>
----------------------	---------------------	-----------------	----------------------

_____ -3 or HP = _____ X _____ = _____

HP = highest number of independent claims paid for, if greater than 3.

3. APPLICATION SIZE FEE


If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is \$270 (\$135 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

<u>Total Sheets</u>	<u>Extra Sheets</u>	<u>Number of each additional 50 or fraction thereof</u>	<u>Fee (\$)</u>	<u>Fee Paid (\$)</u>
_____ -100 = _____	/50 = _____	(round up to a whole number) x _____	_____	_____

4. OTHER FEE(S)

Non-English Specification, \$130 fee (no small entity discount)

Other (e.g., late filing surcharge): Extension of Term Patent**1,120****SUBMITTED BY**

Signature		Registration No. (Attorney/Agent)	44,614	Telephone	206-622-4900
Name (Print/Type)	William T. Christiansen, Ph.D.			Date	May 28, 2009

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: U.S. Patent No. 6,309,650

Inventors: Hyun Su Kim et al.

Issue Date: October 30, 2001

For: ATTENUATED JAPANESE ENCEPHALITIS VIRUS ADAPTED TO VERO
CELL AND A JAPANESE ENCEPHALITIS VACCINE

Assignees: Cheil Jedang Corporation

Government of the United States, as represented by the Secretary of the Army

Date: May 28, 2009

Mail Stop Hatch-Waxman PTE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RECEIVED
MAY 28 2009
PATENT EXTENSION
OPLA

RECEIVED
JUN 02 2009
OFFICE OF PETITIONS

APPLICATION FOR EXTENSION OF PATENT

TERM UNDER 35 U.S.C. §156

Commissioner for Patents:

Applicants, Cheil Jedang Corporation, a corporation organized and existing under the laws of Korea, and having a principal place of business at 500, 5-ga, Namdaemun-ro, Chung-ku, Seoul, 100-095, Korea, and the Government of the United States, as represented by the Secretary of the Army, Walter Reed Army Institute of Research, 503 Robert Grant Avenue, Silver Springs, MD 20910-7500, represent that they are the co-owners of the entire interest in and to U.S. Patent No. 6,309,650, granted to Hyun Su Kim, Wang Don Yoo, Soo Ok Kim, Sung Hee Lee, Sang Bum Moon, Sun Pyo Hong, Yong Cheol Shin, Yong Ju Chung, Kenneth H.

Eckels, Bruce Innis, Joseph R. Putnak, Leonard N. Binn, Ashok K. Srivastava, and Doria R.

Dubois, for "Attenuated Japanese Encephalitis Virus Adapted to Vero Cell and a Japanese Encephalitis Vaccine," as reflected in the assignments recorded by the U.S. Patent and

Trademark Office recorded on October 27, 2000, at Reel 011269, Frame 0694, and December 22, 2000, at Reel 011404, Frame 0913. Attached as **Exhibit A** are copies of the underlying

08/24/2009 RLOGAN 00000001 191090 09406392
01 FC:1457 1120.00 DA

assignment documents. Attached as **Exhibit B** are Power of Attorney documents appointing the undersigned patent attorney as legal representative of Cheil Jedang Corporation.

VaccGen International LLC, a corporation organized and existing under the laws of New York, and having a principal place of business at 8 Cambridge Court, Larchmont, NY 10538 (hereinafter "VaccGen") is the exclusive licensee of U.S. Patent No. 6,309,650 from Applicants. Intercell AG, a corporation organized and existing under the laws of Austria, and having a principal place of business at Campus Vienna Biocenter 3, 1030 Vienna, Austria (hereinafter "Intercell"), is the exclusive sublicensee of U.S. Patent No. 6,309,650 from VaccGen. Intercell is also the owner of a Biologics License Application ("BLA") for IXIARO®, BLA number 125280/0. Attached as **Exhibit C** is a document executed by a duly authorized representative of Vaccgen authorizing Applicants to rely upon activities undertaken by VaccGen during the regulatory review period of IXIARO® for purposes of filing and prosecuting an application to extend the term of U.S. Patent No. 6,309,650 pursuant to 35 U.S.C. § 156, 37 C.F.R. § 1.730, and MPEP § 2752. Attached as **Exhibit D** is a document executed by duly authorized representatives of Intercell authorizing Applicants to rely upon activities undertaken during the regulatory review period of IXIARO® by Intercell and Intercell's subsidiaries, including Intercell Biomedical, Ltd., for purposes of filing and prosecuting an application to extend the term of U.S. Patent No. 6,309,650 pursuant to 35 U.S.C. § 156, 37 C.F.R. § 1.730, and MPEP § 2752.

Applicants, acting through their duly authorized attorney, hereby submit this application for extension of patent term under 35 U.S.C. §156, based upon the approval by the Food and Drug Administration for commercial marketing or use of Japanese Encephalitis Virus Vaccine, Inactivated, Adsorbed Suspension ("IXIARO®"), since the active ingredient of IXIARO® is an inactivated, attenuated Japanese encephalitis virus, which falls within the ambit of the claims of U.S. Patent No. 6,309,650. The information contained in this Application and its Exhibits is provided in accordance with the rules promulgated by the U.S. Patent and Trademark Office at 37 CFR §§1.710-1.785 and presented in the manner set forth at 37 CFR §1.740.

1. A Complete Identification Of The Approved Product As By Appropriate Chemical And Generic Name, Physical Structure Or Characteristics

The approved product, Japanese Encephalitis Virus Vaccine, Inactivated, Adsorbed ("IXIARO®") is a sterile suspension for intramuscular injection. Each dose contains approximately 6 mcg of purified, inactivated Japanese Encephalitis Virus proteins and 250 mcg of aluminum hydroxide. IXIARO® is a vaccine prepared by propagating JEV strain SA₁₄₋₁₄₋₂ in Vero cells. This JEV strain contains the same characteristic amino acid mutations present in the Vero-adapted strain CJ50003 described in U.S. Patent No. 6,309,650; thus, this strain is the basis of the approved product, IXIARO®. Multiple viral harvests are performed, which are pooled, clarified and concentrated. The virus suspension is treated with protamine sulfate to remove contaminating DNA and proteins. The resulting partially purified virus is processed through a sucrose density gradient centrifugation step and fractionated. Each fraction is analyzed for the presence of virus, and fractions with the highest virus activity are pooled to give a purified virus suspension. The purified virus is then inactivated by treatment with formaldehyde. The preparation is adjusted to a specified protein concentration and formulated by addition of aluminum hydroxide. The formulated bulk vaccine is filled into syringes, at a volume of 0.5 ml per syringe. From the manufacturing process, IXIARO® also contains: formaldehyde (not more than 200 ppm), bovine serum albumin (not more than 100 ng/mL), host cell DNA (not more than 200 pg/mL), sodium metabisulphite (not more than 200 ppm), host cell proteins (not more than 300 ng/mL), and protamine sulfate (not more than 1 µg/mL). No preservatives, stabilizers, or antibiotics are added to the formulation.

See the Approved Label attached as **Exhibit E** with regard to the statements in this Section (1).

2. A Complete Identification Of The Federal Statute Including The Applicable Provisions Of Law Under Which The Regulatory Review Occurred

The approved product, IXIARO®, was subject to regulatory review under Section 351 of the Public Health Service Act (42 U.S.C. § 201 *et seq.*) and Section 505 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. § 355 *et seq.*).

3. An Identification Of The Date On Which The Product Received Permission For Commercial Marketing Or Use Under The Provision Of Law Under Which The Applicable Regulatory Review Period Occurred

The approved product, IXIARO®, received permission for commercial marketing or use under Section 351 of the Public Health Service Act (42 U.S.C. § 262) on March 30, 2009. A copy of a letter from the Food and Drug Administration (“FDA”) indicating the date of approval is attached hereto as **Exhibit F**.

4. In The Case Of A Drug Product, An Identification Of Each Active Ingredient In The Product And As To Each Active Ingredient, A Statement That It Has Not Been Previously Approved For Commercial Marketing Or Use Under The Federal Food, Drug, and Cosmetic Act, The Public Health Service Act, Or The Virus-Serum-Toxin Act, Or A Statement Of When The Active Ingredient Was Approved For Commercial Marketing Or Use (Either Alone Or In Combination With Other Active Ingredients), The Use For Which It Was Approved, And The Provision Of Law Under Which It Was Approved

The active ingredient in IXIARO®, is an inactivated, attenuated Japanese encephalitis virus adapted to grow in Vero cells. The Japanese encephalitis virus strain that is the basis of IXIARO® is CJ50003, which has the characteristic amino acid mutations shown in the right hand column of Table 1 of U.S. Patent No. 6,309,650.

The active ingredient of IXIARO® has not been previously approved for commercial marketing or use under the Federal Food, Drug, and Cosmetic Act, the Public Health Service Act, or the Virus-Serum-Toxin Act. Another Japanese encephalitis vaccine, JE-VAX®, was previously approved by the FDA, but this vaccine contains a different active ingredient, since, *inter alia*, the Japanese encephalitis virus used to produce JE-VAX® is the Nakayama strain, which is different from the SA₁₄-14-2/CJ5003 strain used to produce IXIARO®, and the JE-VAX® virus is wild-type (virulent), while the IXIARO® virus is attenuated.

IXARIO® was approved for the prevention of disease caused by Japanese encephalitis virus in persons 17 years of age and older.

IXARIO® was approved under the provisions of Section 351(a) of the Public Health Service Act.

5. A Statement That The Application Is Being Submitted Within The Sixty Day Period Permitted For Submission Pursuant to 37 CFR §1.720(f) And An Identification Of The Date Of The Last Day On Which The Application Could Be Submitted

This application is being submitted within the permitted sixty (60) day period, the last day of which is May 28, 2009.

6. A Complete Identification Of The Patent For Which An Extension Is Being Sought By The Name Of The Inventor, The Patent Number, The Date Of Issue, And The Date Of Expiration

The complete identification of the patent for which extension is sought is:

Inventors: Hyun Su Kim
Wang Don Yoo
Soo Ok Kim
Sung Hee Lee
Sang Bum Moon
Sun Pyo Hong
Yong Cheol Shin
Yong Ju Chung
Kenneth H. Eckels
Bruce Innis
Joseph R. Putnak (as corrected)
Leonard N. Binn
Ashok K. Srivastava
Doria R. Dubois

Patent Number: 6,309,650

PCT Filing Date: August 25, 1998

Issue Date: October 30, 2001

Expiration Date: August 25, 2018 (without extension under 35 U.S.C. §156)

7. A Copy Of The Patent For Which An Extension Is Being Sought, Including The Entire Specification (Including Claims) And Drawings

A copy of U.S. Patent No. 6,309,650 is annexed as **Exhibit G**.

8. A Copy Of Any Disclaimer, Certificate of Correction, Receipt Of Maintenance Fee Payment, Or Reexamination Certificate Issued In The Patent

The patent for which extension is being sought has not been the subject of any disclaimer or reexamination certificate.

A Certificate of Correction was issued on February 10, 2004, a copy of which is annexed as **Exhibit H**.

Maintenance fees were paid on April 6, 2005 and April 1, 2009. Copies of maintenance fee statements showing timely payment of these fees are annexed as **Exhibit I**.

9. A Statement That The Patent Claims The Approved Product Or A Method Of Using Or Manufacturing The Approved Product, And A Showing Which Lists Each Applicable Patent Claim And Demonstrates The Manner In Which Each Applicable Patent Claim Reads On The Approved Product Or Method Of Using Or Manufacturing The Approved Product

U.S. Patent No. 6,309,650 claims the approved product, IXIARO®. More specifically, claims 1 and 8 read on the active ingredient, an inactivated, attenuated Japanese encephalitis virus adapted to grow in Vero cells, and claims 2-5, 9, 10, and 12 read on the final product, a vaccine containing the active ingredient and pharmaceutically acceptable additives.

As described in the Approved Label (**Exhibit E**), the approved product, IXIARO®, contains purified, inactivated JEV virus strain SA₁₄₋₁₄₋₂ propagated in Vero cells, and adjuvant aluminum hydroxide. The approved product, IXIARO®, is prepared by propagating the virus in Vero cells, harvesting, pooling, and concentrating the virus, partially purifying and fractionating the virus by sucrose density gradient centrifugation, and pooling the fractions with the highest virus activity to give a purified virus suspension. The purified virus is then inactivated by treatment with formaldehyde and formulated by addition of aluminum hydroxide. The Vero cell-adapted virus present in the approved product, IXIARO®, contains the same characteristic amino acid mutations present in the Vero-adapted strain CJ50003 described in Table 1 of U.S. Patent No. 6,309,650; thus, the strain which is the basis of the approved product, IXIARO®, is CJ50003.

These claims are compared to the approved product below.

Patent Claim	Approved Product
1. An attenuated Japanese encephalitis virus adapted to Vero cells by passages on Vero cell wherein said virus has a multiplicity of more than 1×10^7 PFU/ml in Vero cells and LD ₅₀ /pfu for young adult mouse is less than 0.000001.	The active ingredient in IXIARO® is an attenuated Japanese encephalitis virus adapted to grow in Vero cells by passage on Vero cells, which has a multiplicity of more than 1×10^7 PFU/ml in Vero cells. The Japanese encephalitis virus strain that is the

	basis of IXIARO® is CJ50003, having the characteristic amino acid mutations shown in the right hand column of Table 1 of U.S. Patent No. 6,309,650, which has an LD ₅₀ /pfu for young adult mouse less than 0.000001, as shown in Table 2 of U.S. Patent No. 6,309,650.
2. A Japanese encephalitis vaccine comprising the attenuated Japanese encephalitis virus according to claim 1.	The approved product, IXIARO®, is a Japanese encephalitis vaccine containing an attenuated Japanese encephalitis virus adapted to Vero cells by passages on Vero cells, wherein said virus has a multiplicity of more than 1x10 ⁷ PFU/ml in Vero cells and an LD ₅₀ /pfu for young adult mouse of less than 0.000001.
3. The vaccine according to claim 2, which further comprises pharmaceutically acceptable additives.	The approved product, IXIARO®, also contains the pharmaceutically acceptable additive, aluminum hydroxide.
4. The vaccine according to claim 2 wherein the virus is inactivated by an inactivating agent.	The virus in the approved product, IXIARO®, is inactivated using formaldehyde.
5. The vaccine according to claim 4, which further comprises pharmaceutically acceptable additives.	The approved product, IXIARO®, contains virus inactivated using formaldehyde and the pharmaceutically acceptable additive, aluminum hydroxide.
8. An attenuated Japanese encephalitis virus adapted to Vero cell by passages on Vero cell which is CJ50003.	The active ingredient in IXIARO® is an attenuated Japanese encephalitis virus adapted to grow in Vero cells by passage on Vero cells. The strain which is the basis of IXIARO® is strain CJ50003, having the characteristic amino acid mutations shown in the right hand column of Table 1 of U.S. Patent No. 6,309,650.
9. A Japanese encephalitis vaccine comprising the attenuated Japanese encephalitis virus according to claim 8.	The approved product, IXIARO®, is a Japanese encephalitis vaccine containing an attenuated Japanese encephalitis virus adapted to Vero

	cells by passages on Vero cells, based on strain CJ50003.
10. The vaccine according to claim 8, wherein the virus is inactivated by an inactivating agent.	The virus in the approved product, IXIARO®, is inactivated using formaldehyde.
12. The vaccine according to claim 9, which further comprises pharmaceutically acceptable additives.	The approved product, IXIARO®, also contains the pharmaceutically acceptable additive, aluminum hydroxide.

U.S. Patent No. 6,309,650 does not claim a method of using or manufacturing the approved product, IXIARO®.

10. A Statement, Beginning On A New Page, Of The Relevant Dates And Information Pursuant To 35 U.S.C. § 156(g) In Order To Enable The Secretary Of Agriculture, As Appropriate, To Determine The Applicable Regulatory Review Period As Follows (i): For A Patent Claiming A Human Drug Product, Antibiotic, Or Human Biological Product, The Effective Date Of The Investigational New Drug (IND) Application And The IND Number; The Date On Which A New Drug Application (NDA) Or A Product License Application (PLA) Was Initially Submitted And The NDA Or PLA Number And The Date On Which The NDA Was Approved Or The Product License Issued

(A) The IND Application (IND 8589) for IXIARO® was submitted to the FDA on September 9, 1999. A copy of the form accompanying the IND submission and indicating the filing date of September 9, 1999 is attached as **Exhibit J**. Accordingly, Applicants believe that the IND effective date is October 9, 1999, which falls 30 days from the IND filing date.

(B) The BLA for IXIARO® was initially submitted to the FDA on December 20, 2007, and assigned Submission Tracking Number (STN) BLA 125280/0. A copy of the letter from the FDA indicating receipt of the BLA is annexed as **Exhibit K**.

(C) The BLA for IXIARO® was approved by the FDA on March 30, 2009. A copy of the approval letter is annexed as **Exhibit F**.

11. A Brief Description Beginning On A New Page Of The Significant Activities Undertaken By The Marketing Applicant During The Applicable Regulatory Review Period With Respect To The Approved Product And The Significant Dates Applicable To Such Activities

A brief description of significant activities undertaken by the marketing applicant during the regulatory review period with respect to the approved product is annexed as **Exhibit L**, the disclosure of which is incorporated in its entirety. A summary of the development of the approved product (referred to therein as “IC51”) is annexed as **Exhibit M**.

Applicant reserves the right to supplement the chronology of **Exhibit L** with materials from which it was derived or other evidence related to Applicant’s conduct in obtaining the approval of IXIARO® *See, e.g.*, 21 CFR §60.32.

12. A Statement Beginning On A New Page That In The Opinion Of The Applicant The Patent Is Eligible For The Extension And A Statement As To The Length Of The Extension Claimed, Including How The Length Of Extension Was Determined

Applicant is of the opinion that U.S. Patent No. 6,309,650 is eligible for extension under 35 U.S.C. § 156, because it satisfies all of the requirements for such extension as follows:

a. 35 U.S.C. §156(a); 37 CFR §1.720(a)

U.S. Patent No. 6,309,650 claims the approved product, as detailed in Section (9) above.

b. 35 U.S.C. §156(a)(1); 37 CFR §1.720(g)

The term of U.S. Patent No. 6,309,650 has not expired before submission of this application.

c. 35 U.S.C. §156(a)(2); 37 CFR §1.720(b)

The term of U.S. Patent No. 6,309,650 has never previously been extended under 35 U.S.C. §156.

d. 35 U.S.C. §156(a)(3); 37 CFR §1.730

This application for extension is submitted by the authorized agent or the owners of record in accordance with the requirement of 35 U.S.C. §156(d) and the rules of the U.S. Patent and Trademark Office.

e. 35 U.S.C. §156(a)(4); 37 CFR §1.720(d)

The product IXIARO® has been subject to a regulatory review period as defined in 35 U.S.C. §156(g) before its commercial marketing or use.

- f. 35 U.S.C. §156(a)(5)(A); 37 CFR §1.720(e)(i)

The commercial marketing or use of the product IXIARO® after the regulatory review period is the first permitted commercial marketing or use of the product under the provision of the Public Health Service Act (42 U.S.C. § 262) under which such regulatory review period occurred.

- g. 35 U.S.C. §156(c)(4); 37 CFR §1.720(h)

No other patent has been extended for the same regulatory review period for the product IXIARO®.

- h. 35 U.S.C. §156(d)(1); 37 CFR §1.720(f)

This application is submitted within the permitted 60 day period beginning on the date the product first received permission for commercial marketing or use.

Applicant is of the opinion that U.S. Patent No. 7,057,053 is eligible for extension under 35 U.S.C. § 156 for 1588 days, as determined pursuant to 37 CFR §1.775 as follows:

Patent Information:

Patent Issue Date	October 30, 2001
Earliest non-provisional priority date	August 25, 1998
Days Extension under 35 U.S.C. 154(b)	0
Original Expiration Date	August 25, 2018

FDA Information:

IND Effective Date	October 9, 1999
BLA Submission Date	December 20, 2007
BLA Approval Date	March 30, 2009

IND Review Period:

The IND Review Period is calculated as the number of days including and following the IND Effective Date and prior to the BLA Submission Date. The Allowed IND Review Period is the IND Review Period less the number of days within the IND Review Period that occurred prior to the Patent Issue Date.

IND Effective Date	October 9, 1999
BLA Submission Date	December 20, 2007
IND Review Period (days)	2994
Allowed IND Review period (days)	2242
½ Allowed IND Review Period (days)	1121

BLA Review Period:

The BLA Review Period is calculated as the number of days including and following the BLA Submission Date and prior to and including the BLA Approval Date. The entire BLA Review Period occurred after the Patent Issue Date.

BLA Submission Date	December 20, 2007
BLA Approval Date	March 30, 2009
BLA Review Period (days)	467

Regulatory Review Period:

The Regulatory Review Period is calculated as the IND Review Period and the BLA Review Period. The Allowed Review Period is calculated as the Allowed IND Review Period and the BLA Review Period less ½ Allowed IND Review Period.

IND Review Period	2994
Allowed IND Review Period	2242
BLA Review Period (days)	467
Regulatory Review Period (days)	3461
Allowed Review Period (days)	1588

Statutory Limitations:

Patent Expiration Date (20 year term)	August 25, 2018
Expiration under 5 yr extension limitation (Date 1)	August 25, 2023
Expiration under 14 yrs from NDA approval limitation (Date 2)	March 30, 2023
Expiration based upon Allowed Review Period (Date 3)	December 30, 2022
Final Expiration Date (Earliest of Date 1, Date 2, or Date 3)	December 30, 2022
<u>Maximum Extension in Days:</u>	1588

U.S. Patent No. 6,309,650 issued after the effective date of Public Law No. 98-417. As such, the two- or three-year limit of 35 U.S.C. § 156(g)(6)(C) does not apply.

13. A Statement That Applicant Acknowledges A Duty To Disclose To The Commissioner Of Patents And Trademarks And The Secretary Of Health And Human Services Any Information Which Is Material To The Determination Of Entitlement To The Extension Sought

Applicants acknowledge a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services any information which is material to any determinations of entitlement to the extension sought in the Application.

14. The Prescribed Fee For Receiving And Acting Upon The Application For Extension

The prescribed fee pursuant to 37 CFR §1.20(j) for receiving and acting upon this application is to be charged to the Deposit Account of Applicant's undersigned attorney as authorized in the attached letter, which is submitted in triplicate.

15. The Name, Address, And Telephone Number Of The Person To Whom Inquiries And Correspondence Relating To The Application For Patent Term Extension Are To Be Directed

Please address all correspondence to:
William T. Christiansen, Ph.D.
Seed Intellectual Property Law Group
701 Fifth Avenue, Suite 5400
Seattle, WA 98026
Telephone (206) 622-4900
Facsimile (206) 682-6031

16. The Application Papers, Certified As Such

Applicants hereby certify that this application for extension is being filed in triplicate.

17. An Oath Or Declaration

Applicants, through their undersigned patent attorney authorized to practice before the Patent and Trademark Office and who has general authority from the agent or owner to act on behalf of the agent or owner in patent matters, being duly warned that willful false statements are punishable by fine or imprisonment or both under section 1001 of Title 18, United States Code and that willful false statements and the like may jeopardize the validity of this application and the patent to which it relates, states and declares that the following statements made based on his own knowledge are true and that all statements made on information and belief are believed to be true:

- (1) The undersigned is registered to practice before the Patent and Trademark Office and is making this declaration as a patent attorney who has general authority to act on behalf of the applicant in patent matters.
- (2) The undersigned has reviewed and understands the contents of the application being submitted pursuant to this section;
- (3) The undersigned believes the patent is subject to an extension pursuant to 37 C.F.R. § 1.710 in the event of NDA approval and, in the interim, is subject to an extension pursuant to 37 C.F.R. § 1.790;
- (4) The undersigned believes an extension of the length claimed is justified under 35 U.S.C. § 156 and the applicable regulations; and
- (5) The undersigned believes the patent for which extension is being sought meets the conditions for extension of the term of a patent as set forth in 37 C.F.R. § 1.720 in the event of NDA approval, and meets the requirements for an interim extension of a patent set forth in 37 C.F.R. § 1.790.

If this application for extension of patent term is held to be informal, Applicants may seek to have that holding reviewed by filing a petition with the required fee, as necessary, pursuant to 37 C.F.R. §§ 1.181, 1.182 or 1.183, as appropriate, within such time as may be set in any notice that the application has been held to be informal, or if no time is set, within two months of the date on which the application was held informal.

Respectfully submitted,

SEED Intellectual Property Law Group PLLC

A handwritten signature in black ink, appearing to read 'W. T. Christiansen', written over a horizontal line.

William T. Christiansen, Ph.D.

Registration No. 44,614

Attorney for Applicants

WTC:CDL:jto

701 Fifth Avenue, Suite 5400

Seattle, Washington 98104

Phone: (206) 622-4900

Fax: (206) 682-6031

#1386252

Exhibit A



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

AUGUST 17, 2001

PTAS

BURNS, DOANE, SWECKER & MATHIS, L.L.P.
RONALD L. GRUDZIECKI
P.O. BOX 1404
ALEXANDRIA, VIRGINIA 22313-1404

**COPY FOR YOUR
INFORMATION**

UNITED STATES PATENT AND TRADEMARK OFFICE
NOTICE OF RECORDATION OF ASSIGNMENT DOCUMENT

THE ENCLOSED DOCUMENT HAS BEEN RECORDED BY THE ASSIGNMENT DIVISION OF THE U.S. PATENT AND TRADEMARK OFFICE. A COMPLETE MICROFILM COPY IS AVAILABLE AT THE ASSIGNMENT SEARCH ROOM ON THE REEL AND FRAME NUMBER REFERENCED BELOW.

PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT AND TRADEMARK ASSIGNMENT SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR HAVE QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYEE WHOSE NAME APPEARS ON THIS NOTICE AT 703-308-9723. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, ASSIGNMENT DIVISION, BOX ASSIGNMENTS, CG-4, 1213 JEFFERSON DAVIS HWY, SUITE 320, WASHINGTON, D.C. 20231.

RECORDATION DATE: 10/27/2000

REEL/FRAME: 011269/0694
NUMBER OF PAGES: 3

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:

KIM, HYUN SU

DOC DATE: 03/21/2000

ASSIGNOR:

YOO, WANG DON

DOC DATE: 03/21/2000

ASSIGNOR:

KIM, SOO OK

DOC DATE: 03/19/2000

ASSIGNOR:

LEE, SUNG HEE

DOC DATE: 03/21/2000

ASSIGNOR:

MOON, SANG BUM

DOC DATE: 03/19/2000

ASSIGNOR:

HONG, SUN PYO

DOC DATE: 03/21/2000

ASSIGNOR:

SHIN, CHEOL YONG

DOC DATE: 03/21/2000

012679-01
RLG/DMM 03/21/00
BURNS, DOANE, SWECKER &
MATHIS, L.L.P. RECEIVED
2001
AUG 22 2001
DOCKETED

Hwang Mok Park & Ji.

011269/0694 PAGE 2

ASSIGNOR:
CHUNG, YONG JU

DOC DATE: 03/21/2000

ASSIGNEE:
CHEIL JEDANG CORPORATION
500, 5-GA, NAMDAEMUN-RO CHUNG-KU
SEOUL 100-095, REPUBLIC OF KOREA

SERIAL NUMBER: 09486392
PATENT NUMBER:

FILING DATE: 06/15/2000
ISSUE DATE:

REGINA COATES-WHITE, SUPERVISOR
ASSIGNMENT DIVISION
OFFICE OF PUBLIC RECORDS

ASSIGNMENT

WHEREAS We, KIM, Hyun Su of Shindong-A Apt. 32-707, Gil 2-dong Kangdong-ku, Seoul 134-766; YOO, Wang Don of Daerim Apt. 108-1001, Daebang-dong, Dongjak-ku, Seoul 156-020; KIM, Soo Ok of Plaza Apt. 10-601, Garak-dong, Songpa-ku, Seoul 138-160; LEE, Sung Hee of Hankuk Apt. 102-808, Maitan 2-dong, Paldal-ku, Suwon, Kyungkwido 442-372; MOON, Sang Bum of San 522-1, Dukpyungri, Majangmyun, Yicheon, Kyungkwido 467-810; HONG, Sun Pyo of Daejin Apt. 106-502, 339, Jukjunri, Suji-up, Yongin, Kyungkwido 449-840; SHIN, Yong Cheol of Samhogarden Apt. 2-809, Banpo 1-dong, Seocho-ku, Seoul 137-761; CHUNG, Yong Ju of Hyundae Apt. 104-1504, Shindaebang 1-dong, Dongjak-ku, Seoul 156-011, all Korean peoples, have made an invention entitled:

AN ATTENUATED JAPANESE ENCEPHALITIS VIRUS ADAPTED TO VERO CELL AND A JAPANESE ENCEPHALITIS VACCINE

NOW, THEREFORE, in consideration of One Won and other valuable consideration paid to us by Cheil Jedang Corporation, a corporation organized under the laws of Korea and having its principal place of business at 500, 5-ga, Namdaemun-ro, Chung-ku, Seoul 100-095, Korea, the receipt of which is hereby acknowledged, and intending to be legally bound, we do hereby assign unto the said Cheil Jedang Corporation, the entire right, title and interest in and to the said invention;

We further assign to the said Cheil Jedang Corporation the right, to apply for, obtain and maintain in all countries, patent applications for said invention, including the full right to claim for any such application the benefits of any priority rights based on Korean Patent Application Nos. 1997/42001 and 1997/42002.

Signed at Seoul, Korea

Date 21th March, 2000

Kim Hyun Su
KIM, HYUN SU

Date March 21, 2000

Yoo Wang Don
YOO, WANG DON

Date March 19, 2000

Soo Ok Kim
KIM, SOO OK

Date March 21, 2000

Lee Sung Hee
LEE, SUNG HEE

Date Mar. 19, 2000

Sang Bum Moon
MOON, SANG BUM

Date 3. 21. 2000

S. p. Hong
HONG, SUN PYO

Date Mar. 21, 2000

Y. C. Shin
SHIN, YONG CHEOL

Date 03, 21, 2000

Y. J. Chung
CHUNG, YONG JU



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

MARCH 20, 2001

PTAS

U.S. ARMY MEDICAL RESEARCH AND MATERIAL
ELIZABETH ARWINE
ATTN: MCMR-JA
504 SCOTT STREET, FORT DETRICK
FREDERICK, MD 21702-5012



101577933A

UNITED STATES PATENT AND TRADEMARK OFFICE
NOTICE OF RECORDATION OF ASSIGNMENT DOCUMENT

THE ENCLOSED DOCUMENT HAS BEEN RECORDED BY THE ASSIGNMENT DIVISION OF THE U.S. PATENT AND TRADEMARK OFFICE. A COMPLETE MICROFILM COPY IS AVAILABLE AT THE ASSIGNMENT SEARCH ROOM ON THE REEL AND FRAME NUMBER REFERENCED BELOW.

PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT AND TRADEMARK ASSIGNMENT SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR HAVE QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYEE WHOSE NAME APPEARS ON THIS NOTICE AT 703-308-9723. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, ASSIGNMENT DIVISION, BOX ASSIGNMENTS, CG-4, 1213 JEFFERSON DAVIS HWY, SUITE 320, WASHINGTON, D.C. 20231.

RECORDATION DATE: 12/22/2000

REEL/FRAME: 011404/0913
NUMBER OF PAGES: 5

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:

ECKELS, KENNETH H.

DOC DATE: 11/21/2000

ASSIGNOR:

PUTNAK, JOSEPH R.

DOC DATE: 11/21/2000

ASSIGNOR:

BINN, LEONARD N.

DOC DATE: 11/21/2000

ASSIGNOR:

SRIVASTAVA, ASHOK K.

DOC DATE: 11/21/2000

ASSIGNOR:

INNIS BRUCE

DOC DATE: 11/29/2000

ASSIGNOR:

DUBOIS, DORIA

DOC DATE: 12/07/2000

USARMC
STAFF JUDGE ADVOCATE
FORT DETRICK, MD
2001 MAR 27 AM 8:12

011404/0913 PAGE 2

ASSIGNEE:

UNITED STATES ARMY
U.S. ARMY MEDICAL RESEARCH AND
MATERIAL COMMAND
ATTN:MCMR-JA 504 SCOTT STREET
FREDERICK, MARYLAND 21702-5012

SERIAL NUMBER: 09486392
PATENT NUMBER:

FILING DATE: 06/15/2000
ISSUE DATE:

SHARON BROOKS, EXAMINER
ASSIGNMENT DIVISION
OFFICE OF PUBLIC RECORDS

ASSIGNMENT OF INVENTION

For use of this form, see AR 27-60; the proponent agency is OTJAG

Title of Invention: An Attenuated Japanese Encephalitis Virus Adapted to Vero Cells and a Japanese Encephalitis Vaccine
Inventor(s) Kenneth Eckels, Bruce Innis, Joseph Putnak, Leonard Binn, Ashok Srivastava, Doria Dubois

*Application Serial No.: 09/486,392

*Date Oath Executed: _____ *Filing Date: February 28, 2000

(*Data not known at execution may be added for better identification.)

I (We), the undersigned inventor(s), in consideration of the rights of the Government of the United States acquired by virtue of the circumstances under which the above-entitled invention was made, hereby:

1. Assign to the Government of the United States, as represented by the Secretary of the Army, the entire right, title and interest throughout the United States, its Territories, Possessions, and Puerto Rico, in and to the above-entitled invention and application for patent and all Letters Patent issuing thereon, and any continuation, continuation-in-part or division of said application and any reissue or extension of said Letters Patent.

2. Agree to assign to the Government upon its request, title and interest in the invention in those foreign countries in which the Government, within eight months of the filing of the United States application for patent, determines to cause an application to be filed; provided that if the Government determines not to cause an application to be filed in any particular foreign country or fails to make such a determination, within the said eight months, all right, title and interest in the invention in such foreign country shall remain in me (us), subject to a nonexclusive, irrevocable, royalty-free license to the Government in any patent which may issue on the invention in such foreign country, including the power to issue sublicenses for use in behalf of the Government and/or furtherance of the foreign policies of the Government.

3. Agree to provide any further information within my (our) knowledge and to execute any further documents necessary to the prosecution of patent applications on the invention, the prosecution and settlement of interferences and recording of title to patent applications and patents issuing thereon.

Signature of Inventor: _____

(First name)

(Middle initial)

(Last name)

Duty Address: Walter Reed Army Institute of Research, Silver Spring, Maryland 20910-7500

(Locality)

(Country)

(State)

Date: 21 Nov 00

Typed Name of Inventor: KENNETH H. ECKELS

State of: District of Columbia

SS

County of: 11

On the above date KENNETH H. ECKELS known to me to be the individual described in and who executed the foregoing instrument duly appeared before me and acknowledged to me that the same as his own free act and deed.

(SEAL)

(Signature of notary public)

JANELLE M. FORDE
Notary Public District of Columbia
My Commission Expires May 14, 2004

SUPPLEMENTAL SIGNATURE SHEET

For use of this form, see AR 27-60; the proponent agency is OTJAG

Use this form with DA Forms 2873-R and 2874-R when additional signature blocks are needed.

1. ASSIGNOR(s) OR LICENSOR(s) NAME(s)
Kenneth Eckels; Bruce Innis; Joseph Putnak; Leonard Binn; Ashok
Srivastava; Doria Dubois

2. APPLICATION SERIAL NUMBER
09/486,392

3. FILING DATE
February 28, 2000

4. TITLE OF INVENTION
An Attenuated Japanese Encephalitis Virus Adapted to Vero Cells and a Japanese Encephalitis Vaccine

SIGNATURE OF INVENTOR:

Bruce
(First name)

Leonard
(Middle initial)

Innis
(Last name)

DUTY ADDRESS: Walter Reed Army Institute of Research, Silver Spring, Maryland 20910-7500

DATE SIGNED: 7 Dec 2000 INVENTOR'S TYPED NAME: BRUCE INNIS

STATE OF

Penney, Virginia

COUNTY OF

Montgomery

) SS.

On the above date
executed the foregoing instrument duly appeared before me and acknowledged to me that he executed the same as his own
free act and deed.

BRUCE INNIS

known to me to be the individual described in and who

(SEAL)

NOTARY SEAL
Rosemary Stuart, Notary Public
Lower Merion Twp., Montgomery County
My Commission Expires October 25, 2004

Rosemary Stuart
(Signature of notary public)

Commission expires on

October 25, 2004

SIGNATURE OF INVENTOR:

Joseph R. Putnak
(First name)

Putnak
(Middle initial)

Putnak
(Last name)

DUTY ADDRESS: Walter Reed Army Institute of Research, Silver Spring, Maryland 20910-7500

DATE SIGNED: 21 Nov 2000 INVENTOR'S TYPED NAME: JOSEPH R. PUTNAK

STATE OF

District of Columbia

COUNTY OF

11

) SS.

On the above date
executed the foregoing instrument duly appeared before me and acknowledged to me that he executed the same as his own
free act and deed.

JOSEPH R. PUTNAK

known to me to be the individual described in and who

(SEAL)

Janelle M. Ford
(Signature of notary public)

My Commission expires on

JANELLE M. FORD
Notary Public District of Columbia
My Commission Expires May 14, 2004

SUPPLEMENTAL SIGNATURE SHEET

For use of this form, see AR 27-80; the proponent agency is OTJAG

Use this form with DA Forms 2873-R and 2874-R when additional signature blocks are needed.

1. ASSIGNOR(s) OR LICENSOR(s) NAME(s)
Kenneth Eckels; Bruce Innis; Joseph Putnak; Leonard Binn; Ashok
Srivastava; Doria Dubois2. APPLICATION SERIAL NUMBER
09/486,3923. FILING DATE
February 28, 20004. TITLE OF INVENTION
An Attenuated Japanese Encephalitis Virus Adapted to Vero Cells and a Japanese Encephalitis Vaccine

SIGNATURE OF INVENTOR:

Leonard
(First name)N.
(Middle initial)Binn
(Last name)DUTY ADDRESS: Walter Reed Army Institute of Research, Silver Spring, Maryland 20910-7500DATE SIGNED: 21 Nov 2000INVENTOR'S TYPED NAME: LEONARD N. BINN

STATE OF

District of Columbia

COUNTY OF

11

) SS.

On the above date LEONARD N. BINN known to me to be the individual described in and who executed the foregoing instrument duly appeared before me and acknowledged to me that he executed the same as his own free act and deed.

(SEAL)

Janelle M. Jack
(Signature of notary public)
Notary Public District of Columbia
My Commission Expires May 14, 2004
My Commission expires on _____

SIGNATURE OF INVENTOR:

Ashok
(First name)Kumar
(Middle initial)Srivastava
(Last name)DUTY ADDRESS: Walter Reed Army Institute of Research, Silver Spring, Maryland 20910-7500DATE SIGNED: Nov 29, 2000INVENTOR'S TYPED NAME: ASHOK K. SRIVASTAVA

STATE OF

N.J.

COUNTY OF

Bergen

) SS.

On the above date ASHOK K. SRIVASTAVA known to me to be the individual described in and who executed the foregoing instrument duly appeared before me and acknowledged to me that he executed the same as his own free act and deed.

(SEAL)

JANE S. BISHOP
(Signature of notary public)
Notary Public, State of New Jersey
No. 2022088
Qualified in Bergen County
Commission Expires Nov. 20, 2001Jane S. Bishop
(Signature of notary public)

My Commission expires on

Nov. 20, 2001

SUPPLEMENTAL SIGNATURE SHEET

For use of this form, see AR 27-80; the proponent agency is OTJAG

Use this form with DA Forms 2873-R and 2874-R when additional signature blocks are needed.

1. ASSIGNOR(s) OR LICENSOR(s) NAME(s)
Kenneth Eckels; Bruce Innis; Joseph Putnak; Leonard Binn; Ashok
Srivastava; Doria Dubois

2. APPLICATION SERIAL NUMBER
09/486,392

3. FILING DATE
February 28, 2000

4. TITLE OF INVENTION
An Attenuated Japanese Encephalitis Virus Adapted to Vero Cells and a Japanese Encephalitis Vaccine

SIGNATURE OF INVENTOR:

(First name)

(Middle initial)

(Last name)

DUTY ADDRESS: Walter Reed Army Institute of Research, Silver Spring, Maryland 20910-7500

DATE SIGNED: 21 Nov 2000

INVENTOR'S TYPED NAME: DORIA R. DUBOIS

STATE OF

District of Columbia

COUNTY OF

IF

SS.

On the above date
executed the foregoing instrument duly appeared before me and acknowledged to me that he executed the same as his own
free act and deed.

DORIA R. DUBOIS

known to me to be the individual described in and who

(SEAL)

Janelle M. Forde
(Signature) JANELLE M. FORDE
Notary Public District of Columbia
My Commission Expires May 14, 2004

My Commission expires on

SIGNATURE OF INVENTOR:

(First name)

(Middle initial)

(Last name)

DUTY ADDRESS:

DATE SIGNED:

INVENTOR'S TYPED NAME:

STATE OF

COUNTY OF

SS.

On the above date
executed the foregoing instrument duly appeared before me and acknowledged to me that he executed the same as his own
free act and deed.

known to me to be the individual described in and who

(SEAL)

(Signature of notary public)

My Commission expires on

Exhibit B

REVOCAION OF POWER OF ATTORNEY WITH NEW POWER OF ATTORNEY AND CHANGE OF CORRESPONDENCE ADDRESS	Patent Number	6,309,650
	Issue Date	October 30, 2001
	First Named Inventor	Hyun Su Kim
	Art Unit	
	Examiner Name	James House
	Attorney Docket Number	201131.401USPC

I hereby revoke all previous powers of attorney given in the above-identified application.

☐ A Power of Attorney is submitted herewith.

OR

☒ I hereby appoint the practitioners at Seed IP Law Group PLLC, Customer Number: **00500**

☒ Please change the correspondence address for the above-identified application to:

☒ The address associated with Customer Number **00500**

OR

<input type="checkbox"/> Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

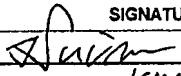
I am the:

☐ Applicant/Inventor.

☒ Assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96)

☒ As assignee of record of the entire interest I/we hereby elect, under 37 CFR 3.71,
to prosecute the application to the exclusion of the inventor(s).

SIGNATURE of Applicant or Assignee of Record

Signature		Date	2009.5.27
Name	KIM, JIN SU		
Title and Company (Assignee)	CEO Chell Jedang Corporation		

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☐ *Total of _____ forms are submitted.

SEND TO: Commissioner for Patents, P.O. Box 1460, Alexandria, VA 22313-1460.

1392264_1.DOC

STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: Hyun Su Kim et al.

Application No./Patent No.: 6,309,650 Filed/Issue Date: October 30, 2001

Entitled: ATTENUATED JAPANESE ENCEPHALITIS VIRUS ADAPTED TO VERO CELL
AND A JAPANESE ENCEPHALITIS VACCINE

Cheil Jedang Corporation

(Name of Assignee)

a

corporation

(Type of Assignee, e.g., corporation, partnership,
university, government agency, etc.)

states that it is:

1. ☒ the assignee of the entire right, title, and interest; or
2. ☐ an assignee of less than the entire right, title and interest.
(The extent (by percentage) of its ownership interest is _____%)

in the patent application/patent identified above by virtue of either:

- A. ☒ An assignment from the inventor(s) of the patent application/patent identified above.
The assignment was recorded in the United States Patent and Trademark Office at
Reel 011269, Frame 0694, or for which a copy thereof is attached.

OR

- B. ☐ A chain of title from the inventor(s), of the patent application/patent identified above, to the
current assignee as follows:

1. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

2. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

3. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet.

- ☒ As required by 37 CFR 3.73(b)(1)(i), the documentary evidence of the chain of title from the
original owner to the assignee was, or concurrently is being, submitted for recordation
pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment
Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP
302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.


Signature

2009. 5. 29
Date

KIM, JIN SU
Typed or printed name

CEO
Title

Exhibit C

VaccGen International LLC

8 Cambridge Court, Larchmont, NY 10538

May 4, 2009

CJ Cheil Jedang Corporation
CJ Bldg. 5001
5-ga, Namdaemun-ro, Jung-gu
Seoul 100-095
Korea

The United States of America
as represented by the Secretary of the Army
Walter Reed Army Institute of Research
503 Robert Grant Avenue
Silver Spring, MD 20910-7500

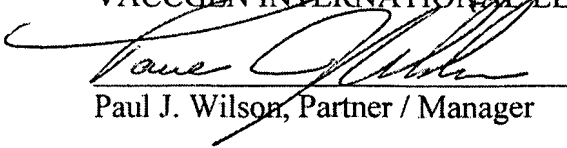
Re: Application for patent term extension of U.S. Pat. No. 6,309,650

Dear Sir or Madam:

VaccGen International LLC, having its principal place of business at 8 Cambridge Court, Larchmont, NY 10538 (hereinafter "VaccGen"), as the exclusive licensee of U.S. Pat. No. 6,309,650 (the "'650 patent") and as sublicensor of the '650 patent to Intercell AG, the marketing applicant for IXIARO[®] (Japanese Encephalitis Vaccine, Inactivated, Adsorbed; biologics license application BLA 125280), hereby authorizes each and both of **Cheil Jedang Corporation** and **The United States of America** as represented by the Secretary of the Army, as record owners of the '650 patent, to rely upon activities undertaken by VaccGen during the regulatory review of IXIARO[®] for purposes of filing and prosecuting an application to extend the term of the '650 patent pursuant to 35 U.S.C. § 156, 37 C.F.R. § 1.730, and MPEP §2752.

Respectfully,

VACCGEN INTERNATIONAL LLC



Paul J. Wilson, Partner / Manager

Cc: Intercell AG

Exhibit D



CJ Cheil Jedang Corporation
CJ Bldg. 5001
5-ga, Namdaemun-ro, Jung-gu
Seoul 100-095
Korea

The United States of America,
as represented by the Secretary of the Army
Walter Reed Army Institute of Research
503 Robert Grant Avenue
Silver Spring, MD 20910-7500

April 29, 2009

Re: **Application for patent term extension of U.S. Pat. No. 6,309,650**

Dear Sir or Madam:

Intercell AG, having its principal place of business at Campus Vienna Biocenter 3, 1030 Vienna, Austria (hereinafter "Intercell"), the marketing applicant for IXIARO® (Japanese Encephalitis Vaccine, Inactivated, Adsorbed; biologics license application BLA 125280), hereby authorizes each and both of **Cheil Jedang Corporation** and **The United States of America** as represented by the Secretary of the Army, as record owners of U.S. Pat. No. 6,309,650 (the " '650 patent"), to rely upon activities undertaken during the regulatory review of IXIARO® by Intercell, the exclusive sublicensee of the '650 patent, and by Intercell's subsidiaries, including Intercell Biomedical, Ltd., for purposes of filing and prosecuting an application for extension of the term of the '650 patent pursuant to 35 U.S.C. §156, 37 C.F.R. §1.730, and MPEP §2752.

Respectfully,

INTERCELL AG

Thomas Lingelbach
COO Intercell AG
CEO, Managing Director Intercell Biomedical, Ltd.

Reinhard Kandra
CFO Intercell AG
Director Intercell Biomedical, Ltd.

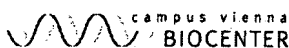


Exhibit E

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use IXIARO safely and effectively. See full prescribing information for IXIARO.

IXIARO (Japanese Encephalitis Vaccine, Inactivated, Adsorbed)

Suspension for Intramuscular Injection

Initial U.S. Approval: 2009

----- INDICATIONS AND USAGE -----

IXIARO is a vaccine indicated for active immunization for the prevention of disease caused by Japanese encephalitis virus (JEV) in persons 17 years of age and older. (1)

----- DOSAGE AND ADMINISTRATION -----

- Immunization consists of 2 doses administered 28 days apart. (2)
- Each 0.5 mL dose is administered intramuscularly. (2)
- Immunization series should be completed at least 1 week prior to potential exposure to JEV. (2, 14)

----- DOSAGE FORMS AND STRENGTHS -----

- Suspension for injection supplied in 0.5 mL single dose syringes. (3, 11)

----- CONTRAINDICATIONS -----

Severe allergic reaction (e.g., anaphylaxis) after a previous dose of IXIARO is a contraindication to administration of IXIARO. (11)

----- WARNINGS AND PRECAUTIONS -----

IXIARO contains protamine sulfate, a compound known to cause hypersensitivity reactions in some individuals. (5, 11)

----- ADVERSE REACTIONS -----

The most common ($\geq 10\%$) systemic adverse events were headache and myalgia. The most common ($\geq 10\%$) injection-site reactions were pain and tenderness (6)

To report SUSPECTED ADVERSE REACTIONS, contact Intercell USA, Inc. at 1-240-454-7333 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

----- USE IN SPECIFIC POPULATIONS -----

Safety and effectiveness have not been established in pregnant women, nursing mothers, or in children and adolescents (younger than 17 years of age). (8.1, 8.2, 8.3)

To report INADVERTANT USE IN PREGNANT WOMEN, contact Intercell USA, Inc. at 1-240-454-7333.

See 17 for PATIENT COUNSELING INFORMATION and FDA-approved patient labeling.

Revised 1/2009

FULL PRESCRIBING INFORMATION: CONTENTS*

- | | | | |
|----------|--|-----------|--|
| 1 | INDICATIONS AND USAGE | | |
| 2 | DOSAGE AND ADMINISTRATION | | |
| 2.1 | Immunization Series | | |
| 2.2 | Administration | | |
| 3 | DOSAGE FORMS AND STRENGTHS | | |
| 4 | CONTRAINDICATIONS | | |
| 5 | WARNINGS AND PRECAUTIONS | | |
| 5.1 | Preventing and Managing Allergic Vaccine Reactions | | |
| 5.2 | Limitations of Vaccine Effectiveness | | |
| 5.3 | Altered Immunocompetence | | |
| 6 | ADVERSE REACTIONS | | |
| 6.1 | Overall Adverse Events | | |
| 6.2 | Clinical Trials Experience | | |
| 7 | DRUG INTERACTIONS | | |
| 7.1 | Use with HAVRIX | | |
| 7.2 | Use with Immunosuppressive Therapies | | |
| 8 | USE IN SPECIFIC POPULATIONS | | |
| 8.1 | Pregnancy | | |
| 8.2 | Nursing Mothers | | |
| 8.3 | Pediatric Use | | |
| | | 8.4 | Geriatric Use |
| | | 11 | DESCRIPTION |
| | | 12 | CLINICAL PHARMACOLOGY |
| | | 12.1 | Mechanism of Action |
| | | 13 | NONCLINICAL TOXICOLOGY |
| | | 13.1 | Carcinogenesis, Mutagenesis, Impairment of Fertility |
| | | 14 | CLINICAL STUDIES |
| | | 15 | REFERENCES |
| | | 16 | HOW SUPPLIED / STORAGE AND HANDLING |
| | | 16.1 | How Supplied |
| | | 16.2 | Storage Conditions |
| | | 16.3 | Handling |
| | | 17 | PATIENT COUNSELING INFORMATION |
| | | 17.1 | Counseling by the Health Care Provider |
| | | 17.2 | FDA-Approved Patient Labeling |

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

IXIARO is a vaccine indicated for the prevention of disease caused by Japanese encephalitis virus (JEV) in persons 17 years of age and older.

2 DOSAGE AND ADMINISTRATION

2.1 Immunization Series

Immunization with IXIARO consists of 2 doses administered 28 days apart. Immunization series should be completed at least 1 week prior to potential exposure to JEV.

2.2 Administration

Before administration, shake the syringe well to obtain a white, opaque, homogeneous suspension. Do not administer if particulate matter remains following shaking or if discoloration is observed.

Each 0.5 mL dose of IXIARO is administered intramuscularly into the deltoid muscle. Do not administer intravenously, intradermally, or subcutaneously.

3 DOSAGE FORMS AND STRENGTHS

IXIARO is a suspension for injection supplied in 0.5 mL single dose syringes. [See Description (11)]

4 CONTRAINDICATIONS

Severe allergic reaction (e.g., anaphylaxis) after a previous dose of IXIARO is a contraindication to administration of IXIARO [See Description (11)].

5 WARNINGS AND PRECAUTIONS

5.1 Preventing and Managing Allergic Vaccine Reactions

IXIARO contains protamine sulfate, a compound known to cause hypersensitivity reactions in some individuals [See Description (11)]. Appropriate medical care should be readily available in case of anaphylactic reaction.

5.2 Limitations of Vaccine Effectiveness

Vaccinees who receive only one dose of IXIARO may have a suboptimal response and may therefore incur higher risk if exposed to JEV compared to vaccinees who receive both doses. Vaccination with IXIARO may not result in protection in all cases. IXIARO will not protect against encephalitis caused by viruses/pathogens other than JEV.

The full duration of protection following immunization is not known [See Clinical Studies (14)]. There are no data regarding timing or efficacy of booster immunization.

5.3 Altered Immunocompetence

There are no safety or efficacy data regarding the use of IXIARO in immunocompromised individuals. Immunocompromised individuals may have a diminished immune response to IXIARO.

6 ADVERSE REACTIONS

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical trials of another vaccine and may not reflect the rates observed in practice.

6.1 Overall Adverse Events

The most common ($\geq 10\%$) systemic adverse events observed in clinical trials with IXIARO were headache and myalgia. The most common ($\geq 10\%$) local reactions after IXIARO administration were pain and tenderness.

In five clinical studies^{1, 2, 3, 4, 5} conducted in North America, Europe, Australia and New Zealand, a total of 3,558 adults aged 18 to 86 years received at least one dose of IXIARO (92% completed the 2 dose series) and were followed-up for safety for at least 6 months after the first dose. In this pooled dataset of subjects who received IXIARO, one death occurred in a subject with metastatic lung adenocarcinoma four months after completing the two-dose regimen. Approximately 1% of subjects who received IXIARO

experienced a serious adverse event, including one case of multiple sclerosis. Approximately 1% of subjects who received IXIARO discontinued due to adverse events.

6.2 Clinical Trials Experience

Adverse Events in a Clinical Trial Comparing IXIARO to a Control:

The safety of IXIARO was evaluated in a randomized, controlled, double-blind clinical trial in healthy male and female subjects¹. IXIARO was compared to a control: Phosphate Buffered Saline containing 0.1% aluminum hydroxide [PBS + Al(OH)₃]. A total of 2,675 subjects were randomized in a 3:1 ratio to receive either an intramuscular injection of IXIARO (0.5 mL) each on Day 0 and Day 28, or an intramuscular injection of PBS + Al(OH)₃ (0.5 mL) each on Day 0 and Day 28. Analysis of safety was carried out using the safety population including 1,993 subjects receiving at least one dose of IXIARO and 657 subjects receiving at least one dose of PBS + Al(OH)₃ (mean age: 33.8 years, range 18 to 86 years; 55.3% female; race: White: 91.7%, Asian: 1.8%, Black: 3.4%, Other: 3.0%). The IXIARO and control groups were similar with regard to demographics. Subjects recorded adverse events on a diary card for the first seven days after each vaccination. In addition, the study investigator took a medical history and performed a physical exam to evaluate for adverse events on the day of each vaccination and at a visit 4 weeks after the second vaccination.

Serious Adverse Events

No deaths occurred during this trial. Sixteen serious adverse events (SAE) were reported during the study period. Ten subjects (0.5%) who received IXIARO and 6 subjects (0.9%) who received PBS + Al(OH)₃ experienced a SAE. The serious adverse events occurring in the IXIARO group were as follows: dermatomyositis, appendicitis, rectal hemorrhage, limb abscess (contralateral to the injected arm), chest pain, ovarian torsion, ruptured corpus luteal cyst, and three orthopedic injuries.

Systemic Adverse Events

Overall, the percentage of subjects who experienced at least one adverse event during the study period was 58.9% in the IXIARO group compared to 56.6% in the PBS + Al(OH)₃ group. The severity of adverse events was as follows: mild in 33.7% of subjects receiving IXIARO compared to 34.1% of subjects receiving PBS + Al(OH)₃, moderate in 20.1% of subjects receiving IXIARO compared to 17.4% of subjects receiving PBS + Al(OH)₃, and severe in 5.1% of subjects receiving IXIARO compared to 5.2% of subjects receiving PBS + Al(OH)₃. Severity was defined as follows: Mild, awareness of signs or symptoms, but easily tolerated; Moderate, discomfort enough to interfere with usual activity; and Severe, incapable of work or usual activity. Adverse events of any severity grade occurring with an incidence of ≥1% of subjects are shown in Table 1.

Table 1. Common Systemic Adverse Events* After IXIARO or Control [PBS + Al(OH)₃], Safety Population

Adverse Event	Incidence (% of subjects) in the First Vaccination Period (Day 0 to Day 28)		Incidence (% of subjects) in the Second Vaccination Period (Day 28 to Day 56)		Incidence (% of subjects) in the Total Vaccination Period (Day 0 to Day 56)	
	IXIARO N _‡ =1993	PBS + Al(OH) ₃ N _‡ =657	IXIARO N _‡ =1968	PBS + Al(OH) ₃ N _‡ =645	IXIARO N _‡ =1993	PBS + Al(OH) ₃ N _‡ =657
Headache†	21.6	20.2	13.4	13.0	27.9	26.2
Myalgia†	13.3	12.9	5.6	5.3	15.6	15.5
Fatigue†	8.6	8.7	5.2	5.9	11.3	11.7
Influenza-like Illness†	8.2	8.5	5.8	4.3	12.3	11.7
Nausea†	4.7	5.3	2.6	3.7	6.6	7.5
Nasopharyngitis	2.3	1.8	2.6	2.3	4.7	4.0
Pyrexia†	1.9	2.1	1.5	1.7	3.2	3.0
Rhinitis	1.0	0.8	0.5	0.6	1.4	1.4
Upper Respiratory Tract Infection	0.9	0.9	0.8	0.9	1.7	2.0
Back Pain	0.8	0.9	0.6	0.2	1.3	1.1
Pharyngolaryngeal Pain	0.8	0.9	1.0	0.5	1.6	1.4
Rash†	0.8	0.9	0.7	0.8	1.3	1.5
Diarrhea	0.8	0.8	0.7	0.3	1.5	1.1
Cough	0.8	0.8	0.6	0.6	1.2	1.2
Vomiting†	0.6	0.8	0.8	0.9	1.4	1.7

*The adverse events in this table are those observed at an incidence of ≥ 1% in the IXIARO or PBS + Al(OH)₃ groups.

† These symptoms were solicited in a subject diary card. Percentages include unsolicited events that occurred after the 7 day period covered by the diary card.

‡N=number of subjects in the safety population (subjects treated with at least one dose) who received the respective dose

Injection Site Reactions

Injection site reactions after IXIARO were compared to reactions after PBS + Al(OH)₃. Symptoms were recorded into a subject diary for the first seven days after each injection, and the injection site was assessed by the investigator at each visit. Pain, tenderness, and pruritis severity was assessed subjectively as absent, mild, moderate or severe by the subject. The amount of erythema, induration and edema was measured by the subject and/or the investigator and rated as absent, mild (≤ 1 cm), moderate (>1 to <3 cm), or severe (≥ 3 cm). The severity of injection site reactions observed after either dose was as follows: mild in 41.6% of subjects receiving IXIARO compared to 44.2% of subjects receiving PBS + Al(OH)₃, moderate in 9.5% of subjects receiving IXIARO compared to 7.7% of subjects receiving PBS + Al(OH)₃, and severe in 3.2% of subjects receiving IXIARO compared to 3.1% of subjects receiving PBS + Al(OH)₃. The frequency of injection site reactions of any severity grade is shown in Table 2.

Table 2. Injection Site Solicited Adverse Reactions* After IXIARO or Control [PBS + Al(OH)₃], Subjects in the Safety Population With Evaluable Diary Cards

Adverse Reaction	Incidence Post Dose 1 (% of subjects†)		Incidence Post Dose 2 (% of subjects†)		Incidence Post Dose 1 or Dose 2 (% of subjects†)	
	IXIARO N‡=1963	PBS + Al(OH) ₃ N‡=645	IXIARO N‡=1951	PBS + Al(OH) ₃ N‡=638	IXIARO N‡=1963	PBS + Al(OH) ₃ N‡=645
Any Reaction	48.5	47.7	32.6	32.2	55.4	56.2
Pain	27.7	28.2	17.7	18.2	33.0	35.8
Tenderness	28.8	26.9	22.5	18.1	35.9	32.6
Erythema	6.8	5.4	4.6	4.1	9.6	7.4
Induration	4.8	5.3	4.0	3.0	7.5	7.4
Edema	2.4	3.3	2.3	1.6	4.2	4.6
Pruritis	2.6	3.3	1.6	1.9	3.8	4.5

* Injection site reactions were assessed for 7 days after each dose.

† Denominators used to calculate percentages are based on the number of evaluable diary card entries (defined as documented presence on *any day* [i.e., entry of “yes”] or absence on *all days* [i.e., entry of “no”]) for each individual symptom and observation period.

‡N=number of subjects who returned diary cards after each dose

Adverse Events in a Clinical Trial Comparing IXIARO to JE-VAX:

The safety of IXIARO compared to another inactivated JE vaccine (JE-VAX) was evaluated in a randomized, double-blind clinical trial².

No deaths occurred during this trial. One serious adverse event occurred in this trial in a subject with a history of myocardial infarction (MI) who experienced a MI three weeks after receiving the 2nd dose of IXIARO. The most common adverse events after immunization occurring in $\geq 1\%$ of subjects were headache, myalgia, fatigue, influenza-like illness, nausea, nasopharyngitis, pyrexia, pharyngolaryngeal pain, cough, rash, diarrhea, sinusitis, upper respiratory tract infection, back pain, migraine, vomiting and influenza, which occurred with similar frequency in both treatment groups. Local injection site reactions solicited in diary cards were observed at a rate of 54% in the IXIARO group (N=428) compared to a rate of 69.1% in the JE-VAX group (N=435).

Safety in Concomitant Use with the Hepatitis A Vaccine, HAVRIX¹

The safety of IXIARO when administered concomitantly with inactivated Hepatitis A Virus vaccine (HAVRIX) was evaluated in a controlled trial in which subjects were assigned randomly to one of three treatment groups: Group A (N=62) received IXIARO + HAVRIX; Group B (N=65) received IXIARO + control [PBS + Al(OH)₃]; Group C (N=65) received HAVRIX + control [PBS + Al(OH)₃]. One serious adverse event occurred in this trial in a subject with a history of alcoholism and seizure disorder who experienced a seizure three weeks after receiving the 2nd dose of IXIARO + control.

The percentage of subjects who experienced at least one adverse event was as follows: Group A: 38.7%; Group B: 41.5%; Group C: 47.7%. The most frequently reported injection site reaction on the day of the first vaccination in all three groups was injection site pain in 59.0% of subjects in Group A, in 48.4% of subjects in Group B and in 48.4% of subjects in Group C.

7 DRUG INTERACTIONS

7.1 Use with HAVRIX

In one clinical trial³, IXIARO was administered concomitantly with HAVRIX (Hepatitis A Vaccine) [See *Adverse Reactions (6)* and *Clinical Studies (14)*]. In this trial, there was no evidence for interference with the immune response to IXIARO or to HAVRIX when HAVRIX was administered concomitantly with dose 1 of IXIARO [See *Clinical Studies (14)*]. Data are not available on concomitant administration of IXIARO with other US-licensed vaccines.

When IXIARO is administered concomitantly with injectable vaccines, they should be given with separate syringes at different injection sites. IXIARO should not be mixed with any other vaccine in the same syringe or vial.

7.2 Use with Immunosuppressive Therapies

There are no data regarding the use of IXIARO concomitantly with immunosuppressive therapies, e.g., irradiation, antimetabolites, alkylating agents, cytotoxic drugs, and corticosteroids (used in greater than physiologic doses) [See *Warnings and Precautions (5)*].

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy category B. Reproduction studies have been performed in female rats at doses approximately 300-fold excess relative to the projected human dose (on a mg/kg basis) and have revealed no evidence of impaired fertility or harm to the fetus due to IXIARO. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, IXIARO should be used during pregnancy only if clearly needed.

The effect of IXIARO vaccine on embryo-fetal and pre-weaning development was evaluated in a developmental toxicity study using pregnant rats. One group of rats was administered IXIARO twice prior to gestation and once during the period of organogenesis (gestation Day 6). A second group of pregnant rats was administered IXIARO once prior to gestation and once during the period of organogenesis (gestation Day 6). IXIARO was administered at 0.5 mL/rat/occasion (approximately 300-fold excess relative to the projected human dose on a mg/kg basis), by intramuscular injection. No adverse effects on mating, fertility, pregnancy, parturition, lactation, embryo-fetal or pre-weaning development were observed. There was a statistically significant finding of incomplete ossification in a few fetuses derived from the second group of pregnant rats. However, there are no data to suggest that this finding is vaccine related. There were no vaccine-related fetal malformations or other evidence of teratogenesis noted in this study.

8.2 Nursing Mothers

It is not known whether this vaccine is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised if IXIARO is administered to a nursing woman.

8.3 Pediatric Use

Safety and effectiveness of IXIARO in a pediatric population (<17 years of age) has not been established.

8.4 Geriatric Use

Clinical studies of IXIARO did not include sufficient numbers of subjects aged 65 years and older to determine whether they respond differently from younger subjects. The limited dataset from the pivotal efficacy study² is as follows: in subjects ≥65 years of age who received IXIARO per protocol (N=24), seroconversion rate was 95.8% and geometric mean titer was 255.2.

In subjects ≥ 65 years of age who had been vaccinated in any of five trials^{1, 2, 3, 4, 5} included in a pooled dataset (N=161), adverse events were reported in 61.9 % (73/118) of subjects in the IXIARO group, 57.7% (15/26) in the JE-VAX group, and 70.6% (12/17) in the control [PBS + Al(OH)₃] group. Five serious adverse events (SAE) were reported. Four subjects (3.4%) who received IXIARO, no subjects who received JE-VAX, and one subject (5.9%) who received the control [PBS + Al(OH)₃] experienced a SAE. The serious adverse events occurring in the IXIARO group were as follows: one case each of rectal hemorrhage, pancreatic adenocarcinoma, breast cancer, and one death in a subject with metastatic lung adenocarcinoma, which occurred four months after the subject completed the two-dose regimen.

11 DESCRIPTION

IXIARO, Japanese Encephalitis Vaccine, Inactivated, Adsorbed is a sterile suspension for intramuscular injection. Each dose of vaccine contains approximately 6 mcg of purified, inactivated JEV proteins and 250 mcg of aluminum hydroxide. The appearance of

the liquid is a white, opaque, non-uniform suspension which becomes homogeneous upon shaking. As IXIARO is inactivated, it cannot cause Japanese Encephalitis.

IXIARO is a vaccine prepared by propagating JEV strain SA₁₄-14-2 in Vero cells. Multiple viral harvests are performed, which are pooled, clarified and concentrated. The virus suspension is treated with protamine sulfate to remove contaminating DNA and proteins. The resulting partially purified virus is processed through a sucrose density gradient centrifugation step and fractionated. Each fraction is analyzed for the presence of virus, and fractions with the highest virus activity are pooled to give a purified virus suspension. The purified virus is then inactivated by treatment with formaldehyde. The preparation is adjusted to a specified protein concentration and formulated by addition of aluminum hydroxide.

The formulated bulk vaccine is filled into syringes, at a volume of 0.5 mL per syringe. From the manufacturing process, IXIARO also contains: formaldehyde (not more than 200 ppm), bovine serum albumin (not more than 100 ng/mL), host cell DNA (not more than 200 pg/mL), sodium metabisulphite (not more than 200 ppm), host cell proteins (not more than 300 ng/mL), and protamine sulfate (not more than 1 µg/mL). No preservatives, stabilizers, or antibiotics are added to the formulation.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Japanese encephalitis is a disease caused by the mosquito-borne Japanese encephalitis virus (JEV). IXIARO acts by inducing antibodies that neutralize live JEV.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

IXIARO has not been evaluated for carcinogenic or mutagenic potential. IXIARO was found to have no effect on fertility of female rats at intramuscular doses of up to 300-fold excess relative to the projected human dose (on a mg/kg basis) administered prior to and after mating [See *Use in Specific Populations* (8.1)]. The effect of IXIARO on male fertility has not been evaluated.

14 CLINICAL STUDIES

Clinical efficacy trials of JE vaccines have found that neutralizing antibody, as measured by a Plaque Reduction Neutralization Test (PRNT) is protective against JEV infection⁶. Therefore, the evaluation of the efficacy of IXIARO was based on immunogenicity using PRNT as a serological correlate of protection. The World Health Organization consultation group recognizes a PRNT titer of $\geq 1:10$ as being a reasonable correlate for protection⁷.

Clinical Trial² of Immunogenicity of IXIARO – Non-inferiority of IXIARO Compared to U.S.-Licensed JE Vaccine, JE-VAX

Immunogenicity of the vaccine was evaluated in a randomized, active-controlled, observer-blinded clinical trial conducted in the U.S., Germany and Austria in 867 healthy male and female subjects 18 to 80 years of age (mean age: 41.3 years; 60.8% female; race: White 80.8%, Asian 0.8%, Black 13.1%, Other 5.3%). Subjects in the IXIARO treatment arm received the following schedule of three intramuscular doses: Day 0, 0.5 mL of IXIARO, Day 7, PBS + Al(OH)₃ (0.5 mL phosphate buffered saline with 0.1% aluminum hydroxide), and on Day 28, 0.5 mL of IXIARO. Subjects in the comparator arm received a subcutaneous dose of 1.0 mL of the US-licensed JEV vaccine, JE-VAX, on Days 0, 7 and 28.

The co-primary endpoints were seroconversion rate (SCR), defined as anti-JEV antibody titer $\geq 1:10$, and geometric mean titer (GMT) at Day 56 in the per protocol population. The immune responses elicited by IXIARO met predefined statistical criteria for non-inferiority compared to those induced by JE-VAX. See Table 3.

Table 3. Seroconversion Rates and Geometric Mean Titers After IXIARO or JE-VAX, Per Protocol Population

Seroconversion Rates			
Time Point	IXIARO SCR (n/N) [95% CI]	JE-VAX SCR (n/N) [95% CI]	Rate difference [95% CI]
Pre-Vaccination Screen	0	0	
Day 56 (28 days after vaccine dose #2)	96.4% (352/365) [94.0, 97.9]	93.8% (347/370) [90.9, 95.8]	2.6% [-0.5, 6.0]†
Geometric Mean Titers			
Time Point	IXIARO N=365 n (GMT) [95% CI's]	JE-VAX N=370 n (GMT) [95% CI's]	GMT ratio estimator [95% CI]
Pre-Vaccination Screen	365 (5.0)∅	370 (5.0)∅	
Day 56 (28 days after vaccine dose #2)	361 (243.6) [216.4, 274.1]	364 (102.0) [90.3, 115.2]	2.33 [1.97, 2.75]‡

†Seroconversion Rates (SCRs): Non-inferiority of IXIARO compared to JE-VAX for SCR was demonstrated if the lower bound of the 2-sided 95% confidence interval (CI) for the SCR difference (IXIARO minus JE-VAX) was > -10% at Day 56.

‡Geometric Mean Titers (GMTs): Non-inferiority of IXIARO compared to JE-VAX for GMTs was demonstrated if the lower bound of the 2-sided 95% CI for the GMT ratio (IXIARO /JE-VAX) was >1/1.5 at Day 56.

∅Pre-Vaccination titers were negative by definition in the PP population and have been imputed to 5.

Follow-up Study of Long Term Immunogenicity (6 months and 12 months)

The persistence of JE-neutralizing antibody was evaluated in a subgroup of subjects recruited for follow-up after participation in one of two clinical trials^{1, 2}. In the Intent-to-Treat (ITT) population of subjects randomized to vaccination with IXIARO (N=181), seroconversion rates (SCR) at 6 and 12 months after initiation of the two-dose series were 95% [95%CI 90.8, 97.4] and 83.4% [95%CI 77.3, 88.1], respectively. Geometric mean titers (GMT) at 6 and 12 months after initiation of the two-dose series were 83.5 [95%CI 70.9, 98.4] and 41.2 [95%CI 34.4, 49.3], respectively.

Temporal Evaluation of Immunogenicity of IXIARO During Vaccination Series

In a randomized, dosing regimen, observer-blinded clinical trial¹ in 374 healthy male and female subjects, aged 18-76 years, the immunogenicity of IXIARO was evaluated on days 10, 28, 35, and 56 during the vaccination period. Seroconversion rates (SCR) at each time point for the subjects randomized to the standard dosing regimen (IXIARO on days 0 and 28) are displayed in Table 4.

Table 4. Seroconversion Rates (SCR) During the Vaccination Series (IXIARO on Days 0 and 28), Per Protocol Population

Day 0 (Dose #1 administered)	Day 10 (10 days post dose #1) SCR (n/N) [95% CI]	Day 28 (28 days post dose #1) SCR (n/N) [95% CI]	Day 28 (Dose #2 administered)	Day 35 (7 days post dose #2) SCR (n/N) [95% CI]	Day 56 (28 days post dose #2) SCR (n/N) [95% CI]
	21.1% (24/114) [13.6%; 28.5%]	39.8% (45/113) [30.8%; 48.8%]		97.3% (110/113) [94.4%; 100.0%]	97.3% (110/113) [94.4%, 100%]

Clinical Trial³ of Immunogenicity of IXIARO and the Hepatitis A Vaccine, HAVRIX, When Used Concomitantly

The concomitant use of IXIARO with inactivated Hepatitis A Virus vaccine (HAVRIX) was evaluated in a randomized, controlled, single-blind clinical trial including 192 healthy male and female subjects aged 18 to 61 years. Subjects were divided into three treatment groups: Group A (N=62) received IXIARO + HAVRIX; Group B (N=65) received IXIARO + control [PBS + Al(OH)₃] (0.5mL phosphate buffered saline with 0.1% aluminum hydroxide by intramuscular injection); Group C (N=65) received HAVRIX + control [PBS + Al(OH)₃]. Anti-JEV GMT at Day 56 in Group A met non-inferiority criteria compared to anti-JEV GMT at Day 56 in Group B. In addition, anti-HAV GMT at Day 28 in Group A met non-inferiority criteria compared to anti-HAV GMT at Day 28 in Group C. Therefore, concomitant administration of IXIARO and HAVRIX did not adversely affect immunogenicity compared to administration of either vaccine individually. Safety results regarding co-administration of IXIARO with HAVRIX are summarized in Section 6.2, Clinical Trials Experience.

15 REFERENCES

1. Clinical study referred to as [NCT00605085](#) in the National Library of Medicine clinical trial database, also referred to as study IC51-302 in the Biologics License Application.
2. Clinical study referred to as [NCT00604708](#) in the National Library of Medicine clinical trial database, also referred to as study IC51-301 in the Biologics License Application.
3. Clinical study referred to as [NCT00596271](#) in the National Library of Medicine clinical trial database, also referred to as study IC51-308 in the Biologics License Application.
4. Clinical study referred to as [NCT00595790](#) in the National Library of Medicine clinical trial database, also referred to as study IC51-304 in the Biologics License Application.
5. Clinical study referred to as [NCT00594958](#) in the National Library of Medicine clinical trial database, also referred to as study IC51-309 in the Biologics License Application.
6. Hoke CH, Nisalak A, Sangawhipa N, Jatanasen S, Laorakapongse T, Innis BL, Kotchasene S, Gingrich JB, Latendresse J, Fukai K, et al. Protection against Japanese encephalitis by inactivated vaccines. *N Engl J Med.* 1988 Sep 8;319(10):608-14.
7. Hombach J, Solomon T, Kurane I, Jacobson J, Wood D. Report on a WHO consultation on immunological endpoints for evaluation of new Japanese encephalitis vaccines, WHO, Geneva, 2-3 September, 2004. *Vaccine.* 2005;23:5205-11.

16 HOW SUPPLIED / STORAGE AND HANDLING

16.1 How Supplied

IXIARO is supplied as a sterile 0.5 mL suspension in a pre-filled syringe (Type I glass) with a plunger stopper (chlorobutyl elastomer) in a pack size of 1 syringe with or without a separate needle. NDC 42515-001-01.

None of the syringe or packaging materials contain latex.

16.2 Storage Conditions

Store in a refrigerator at 2° to 8° C (35° to 46° F). **Do not freeze.**

Do not use the vaccine after the expiration date shown on the label. Store in the original package in order to protect from light. During storage, a clear liquid with a white precipitate can be observed.

16.3 Handling

Prior to agitation, IXIARO may appear as a clear liquid with a white precipitate. After thorough agitation, it forms a white, cloudy liquid/suspension. The vaccine should be visually inspected for coarse particulate matter and discoloration prior to administration. Discard the product if particulates are present or if it appears discolored or if the syringe appears to be physically damaged.

Any unused product or waste material should be disposed of in accordance with local requirements.

17 PATIENT COUNSELING INFORMATION**17.1 Counseling by the Health Care Provider**

Question the vaccine recipient about reactions to previous vaccines, and inform the vaccine recipient of the benefits and risks of IXIARO.

Current U.S. and international advisories should be consulted regarding prevalence of Japanese encephalitis in specific locations. Counsel persons traveling to epidemic and endemic areas that IXIARO may not fully protect everyone who gets the vaccine and that personal precautions should be taken to reduce exposure to mosquito bites (adequate clothing, use of repellents, mosquito nets). When educating vaccine recipients regarding the potential side effects, explain that IXIARO contains formalin-inactivated JEV particles and is, therefore, non-infectious.

Give the required vaccination information to the vaccine recipient, and provide an opportunity to discuss any questions or concerns.

Instruct the vaccine recipient to report any adverse reactions to their health care provider.

17.2 FDA-Approved Patient Labeling

Manufactured by:

Intercell Biomedical

Oakbank Park Road, EH51 0TG, Livingston, UK

T: +44.1506.446.600

F: +44.1506.446.601

www.intercell.com

Distributed by:

Intercell USA, Inc.

20 Firstfield Road

Gaithersburg, MD 20878

US

**Patient Information about
IXIARO (pronounced “ik-sē-ah-rō”)**

Generic name: Japanese encephalitis vaccine, inactivated, adsorbed

Read this information about IXIARO before you are vaccinated. If you have any questions about IXIARO after reading this leaflet, ask your health care provider. This leaflet does not take the place of talking with your health care professional about IXIARO. Only your health care provider can decide if IXIARO is right for you.

What is IXIARO and how does it work?

- IXIARO is a vaccine for use in persons 17 years of age and older to help protect against Japanese encephalitis (JE). You cannot get the disease from IXIARO.
- You will need 2 doses of the vaccine.
- You should still protect yourself from mosquito bites even if you have had the IXIARO vaccine.
- IXIARO may not fully protect everyone who gets the vaccine.
- IXIARO does not protect against encephalitis caused by other viruses/pathogens.
- IXIARO does not protect against other diseases transmitted by mosquitoes.

What is Japanese encephalitis virus (JEV) and what is the disease caused by JEV?

Japanese encephalitis (JE) is caused by the Japanese encephalitis virus, JEV, which is mainly found in Asia. JEV is transmitted to humans by mosquitoes that have bitten an infected animal (like pigs). Many infected people develop mild symptoms or no symptoms at all. In people who develop severe disease, JE usually starts as a flu-like illness, with fever, chills, tiredness, headache, nausea, and vomiting. Confusion and agitation also occur in the early stage. JE causes death in one out of every three people with overt encephalitis. One out of two survivors develops permanent brain damage. JE acquired during pregnancy may cause intrauterine infection and miscarriage.

Who is at risk for Japanese encephalitis?

- People who live in, or travel to, areas where JEV circulates.
- Laboratory personnel who work with JEV.

Who should not get IXIARO?

You should not get IXIARO if you:

- are allergic to any of the ingredients in the vaccine. A list of ingredients can be found at the end of this leaflet.
- had an allergic reaction after getting a dose of the vaccine.

IXIARO is not approved for use in children.

What should I tell my health care professional before I am vaccinated with IXIARO?

It is very important to tell your health care provider if you:

- have had an allergic reaction to a previous dose of IXIARO.
- have a bleeding disorder or a reduction in blood platelets, which increases risk of bleeding or bruising (thrombocytopenia) and cannot receive injections in the arm.
- have a weakened immune system, for example, due to a genetic defect or HIV infection.
- are or may be pregnant, or are breast feeding. IXIARO has not been studied in pregnant women or nursing mothers.
- currently have any illness with a fever of more than 100°F (37.8°C).
- take any medicines, even those you can buy over the counter.

How is IXIARO given?

IXIARO is given as an injection in the upper arm muscle. You will get a total of 2 doses of the vaccine. Ideally, the doses are given as:

- First dose: at a date you and your health care provider choose.
- Second dose: 28 days after the first dose.

Make sure that you get both doses. If you miss the second dose, your health care provider will decide when to give the missed dose. Be aware that maximum protection may not be achieved until 1 week after you receive the second dose of IXIARO.

What are the possible side effects of IXIARO?

The most common side effects are headache, muscle pain and injection site reactions (e.g., pain, swelling, tenderness, redness). Nausea, skin rash, fatigue, flu-like illness, and fever may also occur.

Contact your health care provider right away if you get any symptoms after receiving IXIARO that concern you.

Tell your health care provider if you have any of the following problems because these may be signs of an allergic reaction:

- difficulty breathing
- hoarseness or wheezing
- hives
- dizziness, weakness or fast heart beat

What are the ingredients of IXIARO?

Active Ingredient: purified components of inactivated Japanese encephalitis virus (JEV).

Inactive Ingredients: aluminum hydroxide and phosphate buffered saline (sodium chloride, potassium dihydrogen phosphate, disodium hydrogen phosphate).

Minute amounts of other substances remain in the vaccine as a result of the manufacturing process. Refer to the package insert for a complete list.

What else should I know about IXIARO?

This leaflet is a summary of information about IXIARO. If you would like more information, please talk to your health care professional. U.S. and international agencies (such as cdc.gov and who.int) also provide additional information about JEV and related travel advisories.

Issued January 2009

License Holder:

Intercell AG, Vienna, Austria

Manufacturer:

Intercell Biomedical, Livingston, UK
and

Distributed by:

Intercell USA, Inc., Gaithersburg, MD

Exhibit F

Product Approval Information

March 30, 2009

Our STN: BL 125280/0

Intercell AG
Attention: Paul J. Wilson
Campus Vienna Biocenter 2
1030 Vienna, Austria

Dear Mr. Wilson:

We are issuing Department of Health and Human Services U.S. License No. 1790 to Intercell AG, Vienna, Austria, under the provisions of section 351 (a) of the Public Health Service Act controlling the manufacture and sale of biological products. The license authorizes you to introduce or deliver for introduction into interstate commerce, those products for which your company has demonstrated compliance with establishment and product standards.

Under this license, you are authorized to manufacture **the product *Japanese Encephalitis Virus, Vaccine, Inactivated, Adsorbed***, effective this date. *Japanese Encephalitis Virus Vaccine, Inactivated, Adsorbed*, is indicated for the prevention of disease caused by Japanese encephalitis virus in persons 17 years of age and older.

Under this license, you are approved to manufacture *Japanese Encephalitis Virus Vaccine, Inactivated, Adsorbed*, at Intercell Biomedical Ltd., Livingston, UK. The final formulated product will be manufactured, filled, labeled, and packaged at -----(b)(4)----- (b)(4)----- . You may label your product with the proprietary name, IXIARO ® and it will be supplied and marketed in 0.5 ml mono-dose pre-filled syringes.

The dating period for *Japanese Encephalitis Virus Vaccine, Inactivated, Adsorbed*, shall be 18 months from the date of manufacture when stored at 2-8° C. The date of manufacture shall be defined as the date of filling of the final containers of the formulated drug product. Following the final sterile filtration, no reprocessing/reworking is allowed without prior approval from the Agency. The 18 month shelf life is inclusive of the time that the final filled container is held at 2-8° C prior to packaging. Each unit dose of vaccine contains 6 mcg of the inactivated Japanese encephalitis virus.

Your biologics license application for IXIARO ® was not referred to an FDA advisory committee, because during the BLA review no safety or efficacy issues were identified that required the input of an independent expert panel in order to make an adequate risk benefit assessment.

Please submit final container samples of the product together with lot release protocols in electronic format showing results of all applicable tests. You may not distribute any lots of product until you receive a notification of release from the Director, Center for Biologics Evaluation and Research (CBER).

PEDIATRIC REQUIREMENTS

Under the Pediatric Research Equity Act of 2007 (21 U.S.C. 355c), all applications for new active ingredients, new dosage forms, new indications, new routes of administration, and new dosing regimens are required to contain an assessment of the safety and effectiveness of the product in pediatric patients unless this requirement is waived or deferred.

We are deferring submission of your pediatric studies until Q3/2012 because this product is ready for approval for use in adults and the pediatric studies have not been completed. Your deferred pediatric studies are required under 505B(a) of the Federal Food, Drug, and Cosmetic Act and are therefore required postmarketing studies. The status of these postmarketing studies must be reported according to 21 CFR 601.70 and section 505B(a)(3)(B) of the Federal Food, Drug, and Cosmetic Act. These required studies will be conducted in support of the indication of active immunization for the prevention of disease caused by Japanese encephalitis virus in persons 1 year to 16 years of age. They are listed below:

- 1.I C51-322: IMMUNOGENICITY AND SAFETY OF THE JAPANESE ENCEPHALITIS VACCINE (IXIARO ®) IN A PEDIATRIC POPULATION IN NON-ENDEMIC COUNTRIES. This is an uncontrolled, open-label phase 3 study. 100 subjects aged ≥ 1 to < 17 years will be vaccinated with IXIARO ® to assess the immunogenicity and safety profile in a pediatric population from regions where Japanese encephalitis (JE) is not endemic.

Final Report Submission: Q3/2012

- 2.I C51-323: SAFETY OF THE JAPANESE ENCEPHALITIS VACCINE (IXIARO ®) IN A PEDIATRIC POPULATION. This is an open label, randomized, active controlled Phase 3 study in children aged ≥ 1 to < 17 years of age. 1400 subjects will be randomized to receive IXIARO ® or U.S. licensed Hepatitis A vaccine in a 3:1 ratio. Within each treatment group, the ratio of subjects aged ≥ 1 to < 3 years and ≥ 3 to < 17 years will be approximately 1:1. Subjects randomized into the control group will be offered vaccination against Japanese encephalitis at no cost after concluding the trial.

Final Report Submission: Q3/2012

Submit final study reports to this BLA. For administrative purposes, all submissions related to these required pediatric postmarketing studies must be clearly designated **“Required Pediatric Assessments”**.

We are waiving the pediatric study requirement for ages 0 months to 12 months because the necessary studies would be impossible or highly impracticable. Insufficient numbers of suitable study subjects exist, because (1) maternally derived JE-neutralizing antibodies are common in infants born in endemic areas and (2) non-travelers in non-endemic regions could not be ethically enrolled in a study from which they could not expect any potential benefit.

POSTMARKETING COMMITMENTS SUBJECT TO REPORTING REQUIREMENTS OF 21 CFR 601.70.)

Although clinical data submitted in support of your BLA do not indicate that there are specific safety issues associated with use of IXIARO ® in any age group or gender, you have committed in your e-mail of February 5, 2009, to conduct the following studies according to the following schedule:

Table 1. Timelines for Postmarketing Commitments

Category	Study Code	Protocol to FDA	First Subject Enrolled	Clinical Study Report to FDA
Post-		November/December		

Licensure Elderly	IC51-315	2009	Q2/2010	Q2/2012
Post-licensure surveillance	DoD Active Surveillance in 20,000	September/October 2009*	February/March 2010*	Q3/2012*
Post- Licensure surveillance	DoD Pregnancy Surveillance	September/October 2009*	February/March 2010*	Q4/2013**

* per initial discussions with DoD/Milvax and based on assumptions for supply of IXIARO ® to DoD

**based on follow-up durations for pregnancy cases and children

Please submit the protocols to your IND -(b)(4)-, with a cross-reference to this biologics license application (BLA), STN BLA 125280, and submit all final reports to your BLA, STN BLA 125280.

Please use the following designators to prominently label all submissions, including supplements, relating to these post-marketing study commitments as appropriate:

- **Postmarketing Study Commitment Protocol**
- **Postmarketing Study Correspondence/Status Update**
- **Postmarketing Study Commitment – Final Study Report**
- **Supplement Contains Postmarketing Study Commitments – Final Study Report**

For each postmarketing study subject to the reporting requirements of 21 CFR 601.70, you must describe the status in an annual report on postmarketing studies for this product. Label your annual report “Annual Status Report of Postmarketing Study Commitments.” The status report for each study should include:

- information to identify and describe the postmarketing commitment,
- the original schedule for the commitment,
- the status of the commitment (i.e., pending, ongoing, delayed, terminated, or submitted),
- an explanation of the status including, for clinical studies, the patient accrual rate (i.e., number enrolled to date and the total planned enrollment), and
- a revised schedule if the study schedule has changed and an explanation of the basis for the revision.

As described in 21 CFR 601.70(e), we may publicly disclose information regarding these postmarketing studies on our Web site <http://www.fda.gov/cder/pmc/default.htm>). Please refer to the February 2006 Guidance for Industry: Reports on the Status of Postmarketing Study Commitments – Implementation of Section 130 of the Food and Drug Administration Modernization Act of 1997 (see <http://www.fda.gov/cber/gdlns/post130.htm>) for further information.

You are required to report annually to FDA on the status of this study pursuant to section 506B of the Food Drug and Cosmetics Act, as well as 21 CFR 601.70 until we have notified you that the study commitment has been fulfilled, or that the study would no longer provide useful information.

CMC POSTMARKETING COMMITMENTS

----- (b)(4) -----

ADVERSE EVENT REPORTING

Adverse experience reports should be submitted in accordance with the adverse experience reporting requirements for licensed biological products (21 CFR 600.80) and distribution reports as described in 21 CFR 600.81. (Please see this CBER web site for procedures to submit the distribution reports electronically: <http://www.fda.gov/cber/ldd/ldd.htm>.) Under 21 CFR 600.80(c)(2) [Periodic Adverse Experience Reports], you must report each adverse experience not reported under the paragraph below of this section at quarterly intervals for the first 3 years following approval, and then at annual intervals. Since your product is characterized as a vaccine, submit these reports to the Vaccine Adverse Event Reporting System (VAERS) using the pre-addressed form VAERS-1.

You agree to provide expanded adverse experience reporting (in addition to complying with the requirements under 21 CFR 600.80) to the Vaccine Adverse Reporting System for one year following product licensure as follows:

1. As 15 day reports: All serious adverse events whether expected/labeled or unexpected/unlabeled, including seizures (including febrile seizures), shock, respiratory distress or difficulty breathing, angioedema, inspiratory stridor, anaphylaxis, and bilateral wheezing.
2. As 30 day (monthly) reports: All allergic events, including anaphylaxis not reported as a 15 day report; all cases of urticaria not reported as a 15 day report; neurological events not reported as 15 day reports, including Japanese encephalitis, Bell's palsy, neuritis, multiple sclerosis, CNS inflammation, convulsion, meningitis, Guillain-Barré Syndrome, serum sickness, delayed hypersensitivity, angioedema, encephalitis, encephalopathy, brachial neuritis, optic neuritis, other neuropathy, myelitis including transverse myelitis, ptosis, ataxia, multiple sclerosis, acute disseminated encephalomyelitis, and cerebrovascular accidents or transient ischemic attacks not reported as a 15 day report.

You must submit information to your BLA for our review and written approval under 21 CFR 601.12 for any changes in the manufacturing, testing, packaging, or labeling of IXIARO ® vaccine, or in the manufacturing facilities.

You must submit reports of biological product deviations under 21 CFR 600.14. You should promptly identify and investigate all manufacturing deviations, including those associated with processing, testing, packing, labeling, storage, holding and distribution. If the deviation involves a distributed product, may affect the safety, purity, or potency of the product, and meets the other criteria in the regulation, you must submit a report on Form FDA-3486 to the Director, Office of Compliance and Biologics Quality, Center for Biologics Evaluation and Research, HFM-600, 1401 Rockville Pike, Rockville, MD 20852-1448.

Please submit all final printed labeling and implementation information on FDA Form 356h. Please provide a PDF-format electronic version of the label.

In addition, you may wish to submit two draft copies of the proposed introductory advertising and promotional labeling with FDA Form 2253 to the Center for Biologics Evaluation and Research, Advertising and Promotional Labeling Branch, HFM-602, 1401 Rockville Pike, Rockville, MD 20852-1448. Two copies of final printed advertising and promotional labeling should be submitted at the time

of initial dissemination, accompanied by FDA Form 2253.

All promotional claims must be consistent with and not contrary to approved labeling. You should not make a comparative promotional claim or claim of superiority over other products unless you have submitted data to support such claims to us and received CBER approval for such claims.

If you have any questions, please contact Dr. Richard Daemer or Ms. Daryll Miller at 301-827-3070.

Sincerely yours,

Mary A. Malarkey

Director

Office of Compliance and

Biologics Quality

Center for Biologics

Evaluation and Research

Norman W. Baylor, Ph.D.

Director

Office of Vaccines Research

and Review

Center for Biologics

Evaluation and Research

Updated: March 30, 2009

Exhibit G



US006309650B1

(12) **United States Patent**
Kim et al.

(10) **Patent No.:** **US 6,309,650 B1**
(45) **Date of Patent:** **Oct. 30, 2001**

(54) **ATTENUATED JAPANESE ENCEPHALITIS VIRUS ADAPTED TO VERO CELL AND A JAPANESE ENCEPHALITIS VACCINE**

(75) **Inventors:** **Hyun Su Kim; Wang Don Yoo; Soo Ok Kim**, all of Seoul; **Sung Hee Lee**, Kyungkwido; **Sang Bum Moon**, Kyungkwido; **Sun Pyo Hong**, Kyungkwido; **Yong Cheol Shin; Yong Ju Chung**, both of Seoul, all of (KR); **Kenneth H. Eckels**, Washington, DC (US); **Bruce Innls**, Washington, DC (US); **Joseph R. Punlak**, Washington, DC (US); **Leonard N. Blnn**, Washington, DC (US); **Ashok K. Srivastava**, Washington, DC (US); **Dorla R. Dubols**, Washington, DC (US)

(73) **Assignees:** **Chell Jedang Corporation**, Seoul (KR); **The United States of America as represented by the Secretary of the Army**, Washington, DC (US)

(*) **Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) **Appl. No.:** **09/486,392**

(22) **PCT Filed:** **Aug. 25, 1998**

(86) **PCT No.:** **PCT/KR98/00259**

§ 371 Date: **Jun. 15, 2000**

§ 102(e) Date: **Jun. 15, 2000**

(87) **PCT Pub. No.:** **WO99/11762**

PCT Pub. Date: **Mar. 11, 1999**

(30) **Foreign Application Priority Data**

Aug. 28, 1997 (KR) 97 42001

Aug. 28, 1997 (KR) 97 42002

(51) **Int. Cl.⁷** **A61K 39/12**

(52) **U.S. Cl.** **424/218.1; 424/184.1; 435/235.1; 435/236; 435/237; 435/245**

(58) **Field of Search** **424/184.1, 218.1; 435/5, 41, 173.3, 236, 245, 235.1, 237, 375**

(56) **References Cited**

FOREIGN PATENT DOCUMENTS

0 562 136 9/1993 (EP) .

OTHER PUBLICATIONS

Huiying et al. 1995. The study of adaptation of Japanese encephalitis virus in Vero cells. *Virologica Sinica*. vol. 10. No. 4. See the abstract on p. 277.*

Barret. 1997. Japanese encephalitis and dengue vaccines. *Biologicals*. Mar.; vol. 25, No. 1, pp. 27-34.*

Patent Abstracts of Japan, vol. 14, No. 535, 1990, JP 2-223531, Nov. 26, 1990.

Patent Abstracts of Japan, vol. 13, No. 339, 1989, JP 1-117780, Jul. 31, 1989.

* cited by examiner

Primary Examiner—James Housel

Assistant Examiner—Shanon A. Foley

(74) *Attorney, Agent, or Firm*—Burns, Doane, Swecker & Mathis, L.L.P.; Elizabeth Arwine

(57) **ABSTRACT**

An attenuated Japanese encephalitis virus adapted to Vero cell by passages on Vero cell is disclosed. A Japanese encephalitis vaccine comprising said attenuated virus is also disclosed.

12 Claims, 4 Drawing Sheets

Fig 1.

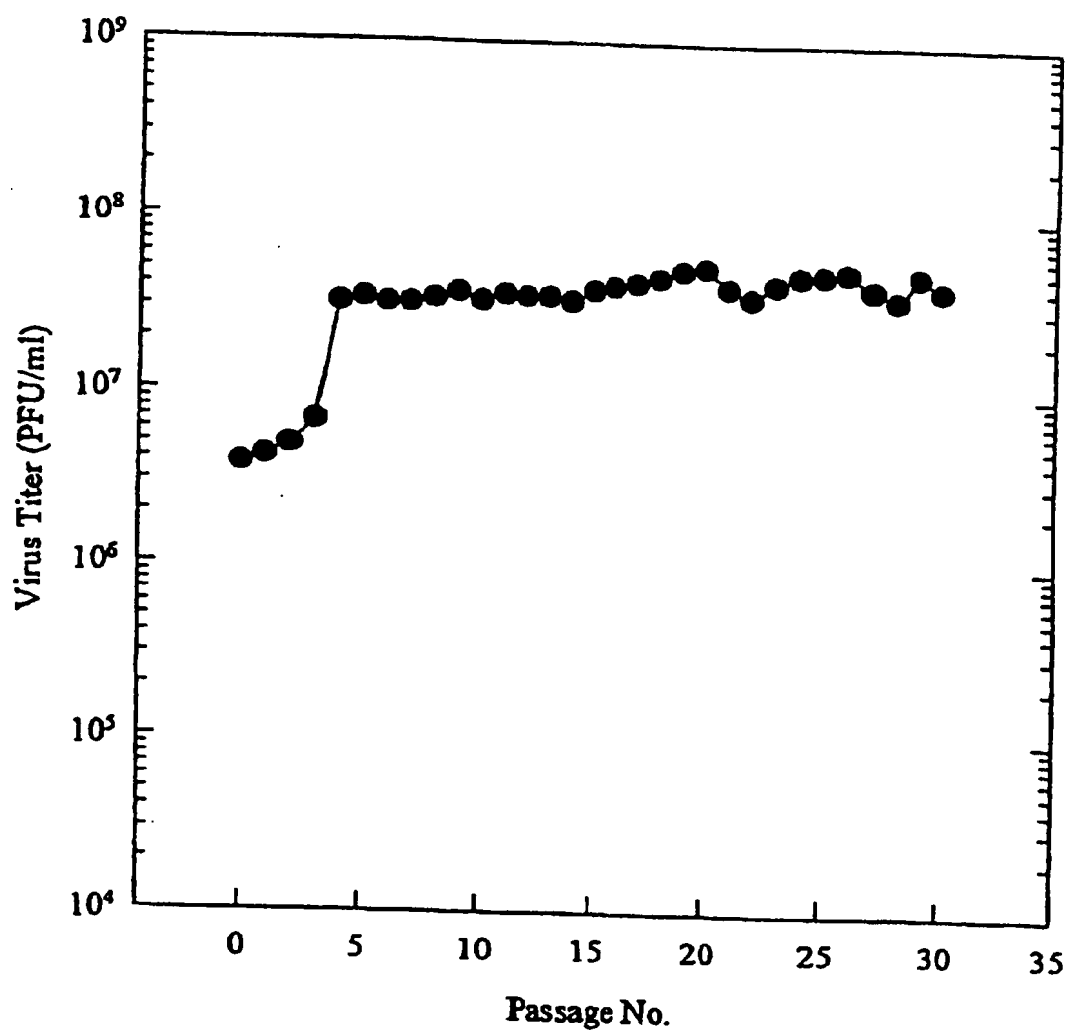


Fig 2.

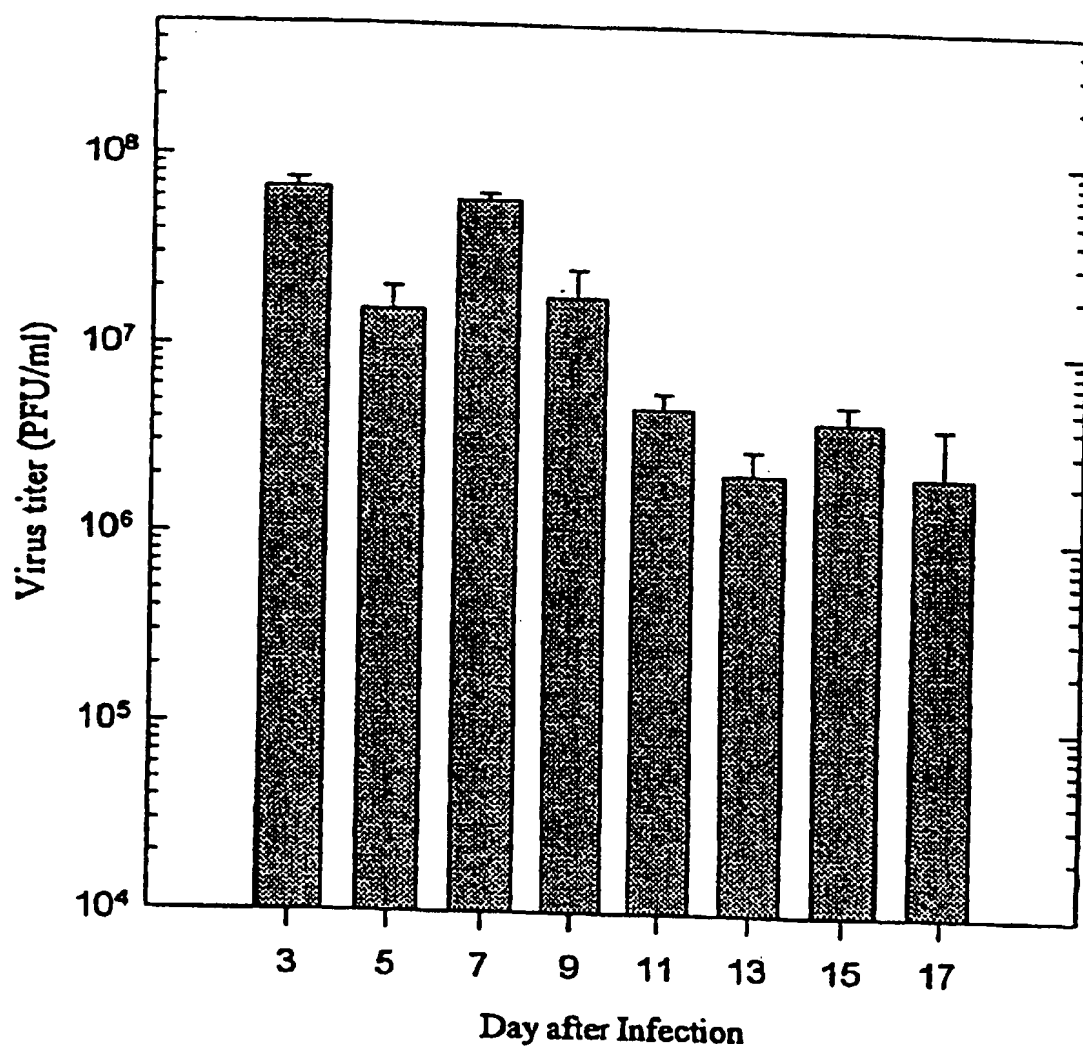


Fig 3.

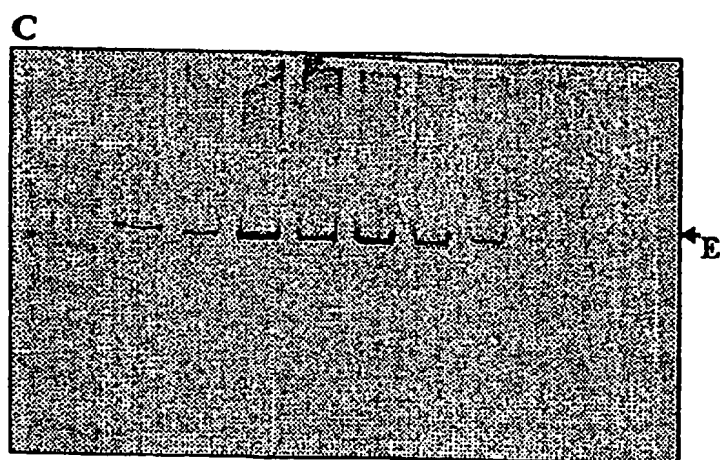
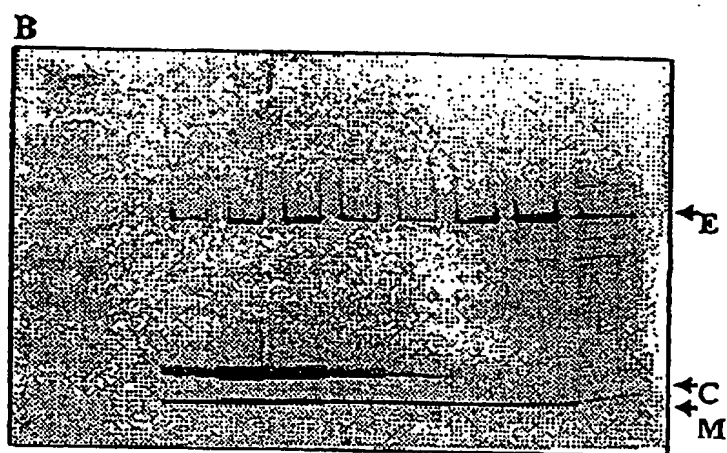
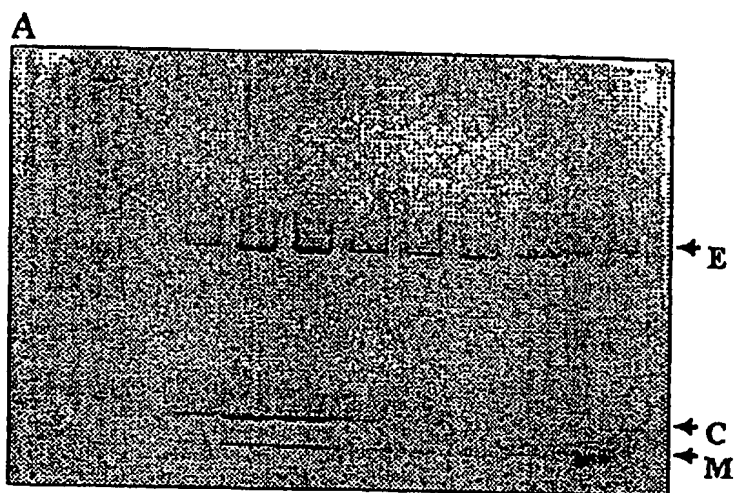
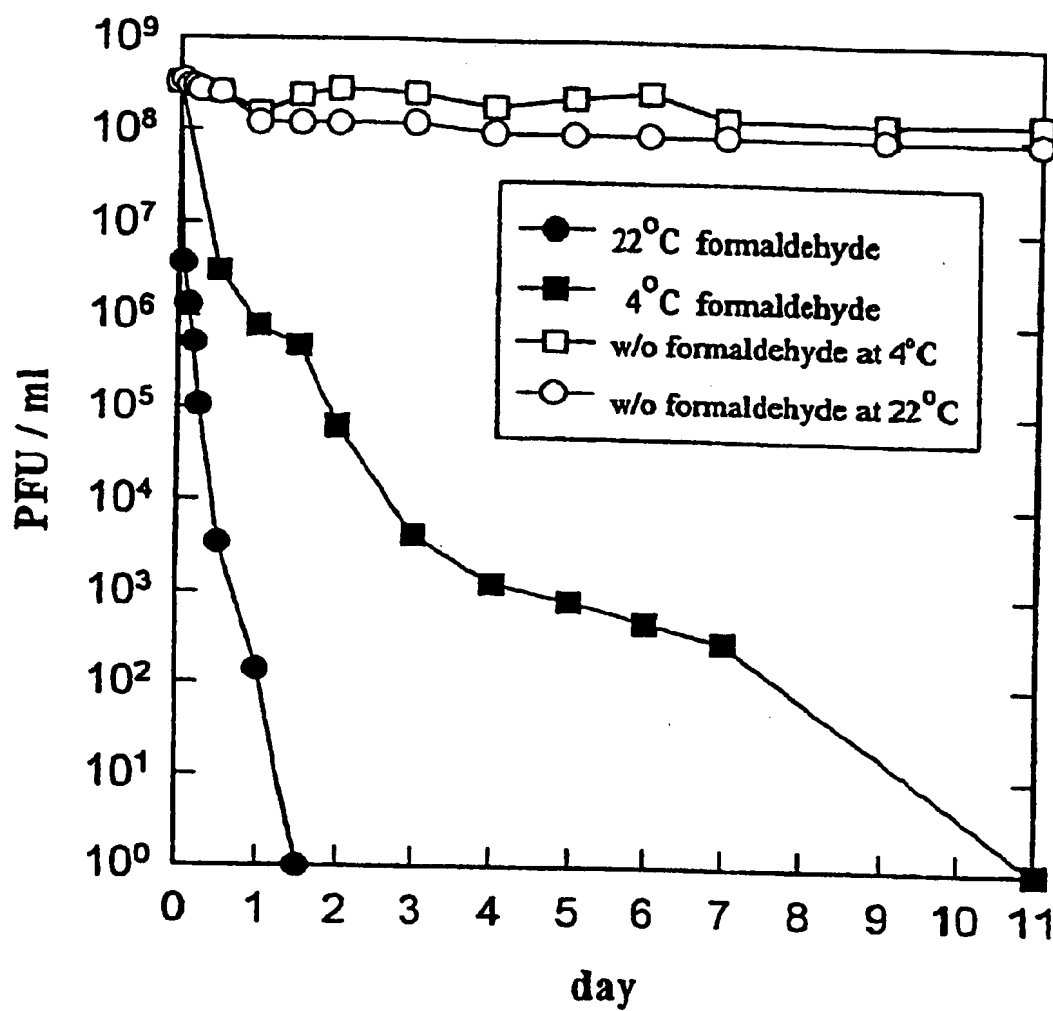


Fig 4.



1

ATTENUATED JAPANESE ENCEPHALITIS VIRUS ADAPTED TO VERO CELL AND A JAPANESE ENCEPHALITIS VACCINE

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to an attenuated Japanese encephalitis virus adapted to Vero cell by passages on Vero cell and a Japanese encephalitis vaccine comprising said attenuated virus.

2. Description of the Prior Art

Japanese encephalitis (JE) is a mosquito-borne arboviral disease of major or public health importance in Asia. More than 35,000 cases and 10,000 deaths are reported annually from that continent, but official reports undoubtedly underestimate the true number of cases (Okuno, T. *World Health Stat Q.* 3: 120-31, 1978; Umenei, T. et al. *Bull World Health Org.* 63: 625-31, 1985). The illness may be manifested by a febrile headache syndrome, aseptic meningitis, or encephalitis and about half of the survivors tend to have permanent neurologic and psychiatric sequelae (Burke, D. S. et al. *The Arbovirus: Epidemiology and Ecology* 3:63-92, 1988; Monath, T. P. *Virology* 763-814, 1990).

JE virus is one of 66 Flaviviridae, enveloped, positive-sense, single stranded RNA viruses that largely are vector-borne (Chambers, T. J. et al. *Ann. Rev. Microbiol.* 44:649-88, 1990). Morphologically, flaviviruses are spherical, approximately 40 nm in diameter, are composed of a lipid bilayer surrounding a nucleocapsid containing 11-kb genome complexed with a capsid (C) protein (Rice, C. M. et al. *Science* 229:726-33, 1985). Surface projections on the membrane are composed of glycosylated envelope (E) and membrane (M) proteins. A pre-M glycoprotein, present in intracellular nascent virions, is cleaved to the M protein, found in mature extracellular virions. Important physiological activities are associated with the 53-kd E protein, including hemagglutination, viral neutralization, virion assembly, membrane fusion, and viral binding to cellular receptors (Koshini, E. et al. *Viol.* 188:714-20, 1992).

There are three JE vaccines for humans (Tsai, T. et al. *Vaccines* 671-713, 1993). Of the three, only inactivated JE vaccine produced in mouse brain is available internationally. One manufacturer, the Research Foundation for Microbial Diseases of Osaka University (Biken) produces most of the inactivated JE vaccine distributed internationally; that vaccine was licensed in 1992 in the USA where it is distributed by Connaught Laboratories, Inc., as JE-VAX™. Inactivated and live attenuated JE vaccine prepared in primary hamster kidney (PHK) cells are distributed solely in China.

Inactivated JE vaccine produced in mouse brains was licensed in Japan in 1954. Because it is produced by cerebral injection of infant mice, it is laborious to manufacture and concerns about the possibility of vaccine-related neurological side effect were raised. Though successive refinements in the manufacturing process have increased its purity and potency (Oya, A. *Vaccination Theory and Practice* 69-82, 1975; Oya, A. *Acta Pediatr Jpn.* 30:175-84, 1988; Takaku, K. Biken J. 11:25-39, 1968), a moderate frequency of local and mild systemic reactions have been reported until recently (Hoke, C. H. et al. *New Engl J Med.* 319:608-14, 1988; Poland, J. D. et al. *J Infect Dis.* 161:878-82, 1990; Sanchez, J. L. et al. *Lancet* 335:972-73, 1990). Local tenderness, redness, and/or swelling at the injection site occur in 20% of vaccines. Mild systemic symptoms, chiefly headache, low-grade fever, myalgias, malaise, and gastrointestinal symptoms, are reported by 10 to 30% of vac-

2

cines. An apparently new pattern of adverse reactions including urticaria, angioedema, respiratory distress, erythema multiforme, erythema nosodum and severe neurological disorders have been reported since 1989, principally among travellers vaccinated in Australia, Europe, and North America (Anderson, M. M. et al. *Lancet.* 337:1044, 1991; Ruff, T. A. et al. *Lancet* 228:881-2, 1991). In addition, in 1992 and 1995 Ohtaki reported seven children with acute disseminated encephalomyelitis (ADEM) with changes on magnetic resonance images (MRI) after JE vaccination (Ohtaki, E. et al. *Pediatr Neurol.* 8:137-9, 1992; Ohtaki, E. et al. *J Neurol Neurosurg Psychiatry* 59:316-7, 1995). Also of note is that vaccination with rabies vaccine containing animal brain tissue has caused severe neurological complications (Plotkin, S. A. et al. *Vaccines* 661-2, 1994). For these reasons, the WHO has designated JE vaccine development as a high priority.

More recently, inactivated and live attenuated JE vaccine of China have proven to be effective, eliciting high titers of virus-neutralizing antibody and conferring solid protection (Tsai, T. et al. *Vaccines* 671-713, 1993). However, PHK cells in which Chinese vaccine were prepared are not approved by the World Health Organization (WHO) for viral vaccine production or licensed for human use by the developed countries. The principal disadvantage in using primary hamster cells for the production of vaccines is the uncertainty with regard to the quality of vaccine. Even if specific pathogen free hamsters are used, animals can unexpectedly become infected, being problematic for vaccine production. Occasionally an infection of this type could be undetected for along time. With these criticisms, further controlled studies of the safety of the vaccine are required to allow confidence regarding its widespread use. Another disadvantage of the vaccine production from primary cells is the low rate of harvest of the virus and high cost without allowing mass production.

In view of the above, there is a need for new JE vaccine which is produced in standard cell lines such as Vero or human diploid cells that have been accepted as human vaccine substrates, with good cost effectiveness. Vero cells are transformed but non-tumorigenic cells derived from monkey kidney. The Vero cell line is more advantageous than any other standard cell line in that Vero cells are more readily adaptable to large scale cell culture and as a transformed cell has an infinite life time.

It has now been found that JE virus can be grown in Vero cell culture. Considerable efforts had been made in the field of JE vaccine to produce vaccine in standard cells which permit effecting cell cultures at a large volume. Nevertheless, virus characterization including genetic stability, yield and process necessary for vaccine commercialization through cultivation with Vero cells had never met the requirements of human vaccine. Owing to these facts and to the difficulties of transposing a knowledge acquired in other virus cultures to JE virus, the prior art had not achieved success in the development of JE virus vaccine which is genetically stable and has a high immunogenic character from continuous cell lines. Among all these researches, none had resulted up to the present time in a new vaccine production which satisfies the criteria mentioned in this background.

The present invention suggests a development and a propagation of JE virus in continuous cell line, Vero cells for vaccine production which overcome previous problems in JE virus produced in mouse brain or primary cell lines. The present invention also identifies methodology developed to cultivate the JE virus and a downstream process for vaccine production with cost-effectiveness.

In addition, the present invention identifies methodology which improves upon the previously commercialized JE vaccines in the following ways.

1. Safety: The invented virus did not acquire the virulence through the Vero cell cultivation, reducing the hazards of production and affording an additional level of safety to recipients beyond that furnished by stringent control over the virus-inactivation process. This advantage has never been provided by the previously commercialized JE vaccines.
2. Increased supply in safer production substrate: The JE vaccine of the present invention is produced in the absence of bovine serum, making high yields and inexpensive and scalable production which are not achieved in the previously commercialized JE vaccines.
3. Less reactogenicity: No gelatin stabilizer is incorporated into the JE vaccine of the present invention, reducing the risk of vaccine reactions like those seen with the existing vaccine (Saskaguchi M. et al. Vaccines 68-69, 1998). In addition, undesirable bovine-derived components, incorporated in the existing JE vaccines are effectively eliminated. Conclusively, this safety point of the present invention has never been provided by the previous JE vaccine.
4. Increased potency: The success of scalable production with Vero cells and the absence of supplements for production, as well as the effective purification, permits the first use of potent adjuvants in formulating the JE vaccine. Although the use of the adjuvants in the vaccine formulation has been applied in other vaccine, transposing this knowledge to the JE vaccine has been in difficulty since none of the existing JE vaccines assure it's safe production.

In conclusion, none had resulted up to the present time in a new vaccine which satisfies the aforementioned advantages in the commercialization of JE vaccine.

Therefore, an object of the present invention is to provide a safe and effective JE vaccine produced in standard cell substrate to increase its acceptability in many countries. A further object of the invention is to provide an effective process for producing a highly purified stable vaccine and formulating a vaccine which has a high immunogenic character with a small antigen amount.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides an attenuated Japanese encephalitis virus adapted to Vero cell by passages on Vero Cell. The attenuated Japanese encephalitis virus of the present invention, which is referred to as CJ50003 herein, was deposited at the permanent collection of the Korean Culture Center of Microorganisms, Seoul, Korea, on Apr. 20, 1998 under the Budapest Treaty of the international recognition of the deposit of microorganisms for the purpose of patent procedure, and a subculture thereof can be obtained from the repository under the accession number KCCM-10125.

In another aspect, the present invention provides a Japanese encephalitis vaccine comprising an attenuated Japanese encephalitis virus adapted to Vero cell by passages on Vero cell.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other features, aspects, and advantages of the present invention will become better understood with regard

to the following description, appended claims, and accompanying drawings as follows:

FIG. 1 shows passages and adaptation of JE virus SA14-14-2(PDK) in Vero cells. Virus passage 1 was harvested at 5 days after inoculation of Vero cell monolayer with 0.1 moi of SA14-14-2(PDK) strain. The JE virus titer was measured by plaque assay performed on Vero cell monolayers. Subsequent serial passages were conducted to 30 passages with virus passage 1 virus as starting material by successive virus infection and titration as described in Example 1.

FIG. 2 shows multiple time harvest of JE virus CJ50003 in a roller bottle every other day from 3 to 17 days post infection. Vero cell monolayer were infected at a moi of 0.01 plaque forming unit (pfu) per cell. Virus was allowed to adsorb for 2 hrs at 35° C., then cells were washed with PBS three times, fed with 100 ml of serum-free EMEM and incubated at 35° C. Every 48 hrs from 3 days to 17 days post inoculation, culture supernatants were replaced with fresh serum-free EMEM. Virus infectivity titrations of the harvests were performed by plaquing on Vero-cell monolayers.

FIG. 3 shows an analysis of JE virus CJ50003 purified by sucrose gradient ultracentrifugation by SDS-PAGE and Western blotting. Sixty ml of concentrated culture supernatant was applied to a forty ml of 15-60% sucrose gradient and centrifuged in a 45 Ti rotor at 22,000 rpm, 18 hrs., 12° C. Two ml samples were collected from the bottom of the tube and subjected to 4-20% gradient SDS-PAGE and the resolved proteins were transferred to Nitrocellulose membranes. Proteins were visualized by staining with Coomassie brilliant blue (Panel A) or silver nitrate (Panel B), and antigens were visualized by reaction with monoclonal antibody reactive against JE viral E protein (Panel C). Lane 1, pre-stained protein standards (Novex Seeblue™) representing molecular weights of 250, 98, 64, 50, 36, 30, 16, and 6 kDa from the top; Lane 2-10, fraction No. 3-11 from the bottom after ultracentrifugation; E, envelope protein; C, capsid protein; M, membrane protein.

FIG. 4 shows formaldehyde inactivation kinetics of purified JE virus CJ50003. Purified JE virus preparations were inactivated with 0.018% formaldehyde at 4° C. or 22° C. Samples taken at the indicated times were titrated for their residual infectious virus by direct plaquing on Vero cell monolayers. Additionally, amplification assay was done to detect low levels of virus as follows. Duplicate flasks containing Vero cell monolayers were inoculated with samples from virus-inactivation time points. After a 2 hr adsorption period at 35° C., cells were refed and incubated at 35° C. Cells were refed at 7 days and at 14 days post-infection. The culture media were harvested and plaqued to detect infectious virus. Inactivation time points from two separate experiments are shown: 4° C. (filled rectangle), 22° C. (filled circle). Thermal inactivation (no formaldehyde) controls (open rectangle for 4° C. and open circle for 22° C.) were done in parallel.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a JE virus strain which has desirable properties for the preparation of the JE vaccine. Said virus is an attenuated virus and can propagate in the continuous cell line, Vero which is admitted by WHO as cell substrate for human vaccine production. Thus it is expected that said virus can be used for the preparation of more safe inactivated and live JE vaccines than current vaccines.

The present invention provides a vaccine that satisfies the present need. A JE virus has been adapted to Vero cell by

serial passages at no higher than 35° C. Continued passages in Vero cell resulted in increase in virus titer, over 10⁷ pfu per ml of culture supernatant and reduced culture time to show peak virus titer. The invention concerns the development of a multiple time harvesting process with no serum requirement as supplement resulted in high yield of virus productivity, which is commercially feasible properties for the large-scale production of said vaccine with cost-effectiveness. According to the invention, the multiple harvesting process in virus cultivation is responsible for the reduced degree of cytopathic effect (CPE) of infected cells. The JE vaccine of the present invention contains an extremely small quantity of residual cell derived components owing to the reduced level of CPE. In addition, JE vaccine of the present invention is expected to afford enhanced immunogenicity and greater protection against disease than current JE vaccines. The purified JE vaccine of the present invention has a major advantage over current vaccines in that the purified viruses from the cultured Vero cells meet the development requirements for human vaccine.

The present invention also relates to the methods for the preparation of said vaccine. The methods can provide high productivity, purity and potency of said vaccine. The JE virus CJ50003 is obtained by subjecting JE virus SA14-14-2(PDK) to 4 passages or more of adapting in the Vero tissue culture cells at temperatures no higher than 35° C. and selecting the cultured virus while monitoring the virus propagation based on the number of foci which were formed in Vero and/or LLC-MK2 cells. The virus obtained from that adaptation has a peak titer of at least 1×10⁷ pfu/ml of culture supernatant in Vero cell culture and reduced incubation period for harvest. The JE virus SA14-14-2 is an attenuated strain which is obtained by adapting a wild type JE virus SA14 from mosquito in the Primary Hamster Kidney (PHK) tissue culture cell and the Primary Dog Kidney (PDK) tissue culture cell (Kenneth H. Eckels, et al. *Vaccine* 6 513-518, 1988). But the PHK and PDK cells are not admitted by WHO, so they are not suitable for preparation of vaccines applicable to humans. The Vero cell is admitted by WHO for human use, so the Vero adapted JE virus strain, CJ50003, is a good basis for production of vaccine for humans.

It is known that SA14-14-2(PDK) virus belongs to flaviviridae and has the following physicochemical properties: single-stranded, positive-sense RNA genome with 5' methylated end and 3' end with no poly A structure. The size of RNA genome is approximately 11 kb and the genome is in a combined state with nucleocapsid (C) protein of 13,500 Da. The virus is additionally comprised of membrane (M) protein of 8,700 Da, envelope (E) protein of 53,000 Da and non-structural proteins NS1, 2a, 2b, 3, 4a, 5 and the like.

The Vero adapted JE virus strain, CJ50003 was passed in Vero cell over 30 passages. The virus titer and the morphology of plaque were not varied through passaging, suggesting that the virus has stable phenotypic character.

To get an insight into the molecular basis for the biological characteristics of JE virus CJ50003, the physicochemical properties of the virus were analyzed. The sequence of the bases of the viral genome was determined by cDNA cloning and sequencing. As a result, it was discovered that three adenine bases of the 1032, 1506, and 1704 positions, and a guanine base of the 1769 position of E protein gene of JE SA14-14-2(PDK) virus were replaced by three guanines and a thymine in JE CJ50003 virus, respectively. Accordingly, the amino acid sequence of E protein was changed from threonine of the 19 position, threonine of the 177 position, lysine of the 243 position and glutamine of the 264 position to alanine, alanine, glutamate and histidine, respectively.

The amino acid changes on the E protein were maintained through passaging the virus in Vero cell as long as our investigation lasted.

The JE virus CJ50003 did not kill mice when the viruses which have different number of passages in Vero cells, were injected to young mice intracerebrally. Accordingly, it can be said that the Vero adapted JE virus CJ50003 is an attenuated and stable virus strain which has no or little neurovirulence. It is one of the critical points to use said virus for the live JE virus vaccine and/or inactivated vaccine.

The present invention also provides a method for purifying virus from cell culture without freezing the crude or interim purity materials. Said method comprises the steps of removing cell debris, concentrating the virus, purifying the virus by precipitation of the materials of cell origin and sucrose gradient ultracentrifugation, fractionating the gradients and assaying the fractions for virus. More specifically, the present invention provides a method for the production of purified JE virus, by propagating virus to high titer in continuous cell lines, in the presence or absence of serum protein supplements, purifying the virus by ultracentrifugation, and pooling the virus-positive fractions.

The said virus is propagated in Vero tissue culture cells. The confluent grown Vero cells in roller bottles are infected and incubated with the CJ50003 virus. Harvesting the virus can be done by the multiple harvesting method. The harvest of culture supernatant was started at the point of 2 or 3 days post infection according to moi of infection, and the fresh medium was refed to the culture. After 2 day incubation of the refed culture, the culture supernatant was harvested again. Harvesting can be repeated up to 4 times by 8 or 9 days post infection with the virus titer maintained over 10⁷ pfu/ml of culture supernatant. The multiple harvesting method gave a high yield of virus per unit roller bottle, so it makes this invention more compatible with the laws of market. Furthermore, the process is responsible for the reduced degree of CPE of infected cells. The reduced level of CPE contributes to extremely low level of residual cell derived components in JE vaccine of the present invention. The harvested culture supernatants can be stored at 4° C. until the purification started. The clarification of the harvested culture supernatants can be accomplished by common methods known in the art including low-speed centrifugation, for example, at 1500 g for 10 min, and/or by filtration through a filter of pore size of 0.45. The harvested culture fluid is stored at 4° C. until concentration. For the concentration of the virus, the culture fluid is ultrafiltrated and the retentate is collected. In another method for concentration, the polyethylene glycol (PEG) 8000 is dissolved in the culture fluid up to 10% and the precipitate is dissolved in a proper buffer, for example phosphate-buffered saline (PBS, pH 7.0). The protamine sulfate precipitation is performed for removing DNA or other materials which originated from the cell, which can be accomplished by addition of protamine sulfate to concentrated virus solution and high speed centrifugation, for example, at 12,000 g, for 5 min. For further purification of the virus, density gradient ultracentrifugation is performed on 15-60% continuous or multi-step sucrose gradients. The sucrose gradient is fractionated and the fractions are assayed for the virus. Methods for assaying for virus positive fractions include plaque assay, hemagglutination assay, polyacrylamide gel electrophoresis, and antigen assays such as immunoassays. The fractions for further processing are pooled on the basis of high virus titer and low level of other impurities. The purity of the pooled purified virus was estimated by testing for Vero cell originated chromosomal DNA and protein. The results showed

that contents of host cellular DNA and protein are as low as 2.5 pg and 2 ng per 5 µg of purified JE virus, respectively, which demonstrated the purification methods described above effectively removed other impurities from viral antigen. JE virus yield from 1 L of infected culture fluid is estimated to be about 2.3 milligrams.

The present invention also provides a method of inactivating JE virus to destroy its infectivity while preserving its antigenicity. Said method comprises adding an effective quantity of formaldehyde and incubating said virus with said agent in certain conditions such that said virus is inactivated. Specifically, the fraction pool was diluted to appropriate protein concentration with a proper buffer such as PBS and the formaldehyde was added to the diluted fraction pool. The incubation with formaldehyde was performed at 22° C. or 4° C. At least 4 or 46 days were required to fully destroy viral infectivity without loss of viral antigenicity at 22° C. or 4° C, respectively. The inactivation process of JE virus at 22° C. was preferably chosen for simplicity in large scale culture and incubation time was extended to 7 days for a safety margin. However, examples of inactivating agents which were effective include but are not limited to formaldehyde. In general, this can be achieved by chemical or physical means. Chemical inactivation can be effected by treating the viruses, for example, with enzymes, β-propionlactone, ethylene-imine or a derivative thereof, and an organic solvent such as Tween, Triton, sodium deoxycholate, and sulphohetain. If necessary, the inactivating substance is neutralized afterwards; material inactivated with formaldehyde can, for example, be neutralized with thiosulphate. Physical inactivation can preferably be carried out by subjecting the viruses to energy-rich radiation, such as UV light, X-radiation or gamma-radiation.

The JE vaccines are prepared as injectables, either as liquid solution or suspension. It is possible to add a stabilizing agent such as carbohydrates (sorbitol, mannitol, starch, sucrose, dextran, glucose, etc), proteins (albumins, casein, etc), an agent containing proteins (bovine serum, skim milk, etc) and buffers (such as alkali metal phosphate). The preparation can be lyophilized after adding a stabilizer and it can be vacuum or nitrogen stored. If desired, one or more compounds with an adjuvant action can be added. Suitable compounds for this purpose are, for example, aluminum hydroxide, phosphate or oxide, mineral oil (such as Bayol, Marcol 52) and saponins. In addition, if desired, one or more emulsifiers, such as Tween and span, is also added to the virus materials.

The effectiveness of an adjuvant was determined by measuring the amount of neutralizing antibodies directed against the virus resulting from administration of the inactivated virus in vaccines which are also absorbed to an adjuvant. Examples of adjuvant which was effective include but is not limited to alum hydroxide. The obtained vaccine was investigated for efficacy by the plaque reduction neutralization test (PRNT) with the sera of said vaccine immunized mice and direct challenge of immunized mice with a neurovirulent virus. As a result, it was shown that the said vaccine had the same as or better efficacy of eliciting neutralizing antibody than comparable vaccines.

To investigate possible changes in immunogenicity of Vero adapted viruses with different passage numbers, the vaccines were prepared in different passage numbers and the efficacy of each vaccine was compared. There was no remarkable difference in efficacy among the vaccines prepared from viruses with different passage numbers in spite of successive passing in Vero cell. Thus it can be said that the Vero adapted JE virus strain, CJ50003, has stable immunogenicity.

The following examples illustrate the attenuated JE virus adapted to Vero cell according to the present invention and the JE vaccine comprising said attenuated virus according to the present invention. From the foregoing description and the following examples, it is believed that those skilled in the art would be able to carry out the invention to the fullest extent.

EXAMPLE 1

Adaptation of SA14-14-2(PDK) Virus in Vero Cell

JESA14-14-2 (PDK), SA14-14-2 virus in dog kidney cell culture passage 8 was used to initiate serial passages in Vero cell culture. The Vero cell monolayers were inoculated with JE SA14-14-2 (PDK) at an moi of 0.1 pfu per cell. The infected cell cultures were grown in 25 cm² culture flasks containing 5 ml of nutrient media consisting of Eagle's minimal essential media supplemented with 10% fetal bovine serum in an atmosphere of about 5% CO₂ in air and at a temperature no higher than about 35° C., typically at from about 32° C. to about 35° C., with about 35° C. being preferred. Viral growth was monitored by microscopic observation of cytopathic effect (CPE) and various assay for the presence of viral antigen including hemadsorption assay (HA), plaque assay, and enzyme linked immunoadsorbant assay (ELISA). JE virus was harvested at day 5 post infection when the culture showed peak of virus titer, clarified by centrifugation. The single plaque was purified from the clarified supernatant and amplified in Vero cells. The amplified virus was re-infected to Vero cell for further passages. Subsequent serial passages were conducted up to 30 passages by successive virus infection, titration, and plaque-purification as described above. As shown in FIG. 1, the virus titers reached about 4×10⁷ pfu per ml of culture supernatant with 4 passages in Vero cells and maintained close to this level in further passages. Besides, the optimal period for viral harvest was reduced from 5 days at passage 1 to 2–3 days at passage 4. A significant increase in virus yield, about 10⁵ pfu/ml to over 10⁷ pfu/ml and a decrease in incubation time resulted in the selection of the JE passage 4 in Vero cells as starting material of choice for the preparation of a candidate JE vaccine. The JE passage 4 in Vero cells was labelled as CJ50003 (Vero, PS4). Abbreviation PS means virus passage number in designated cell.

EXAMPLE 2

Characterization of CJ50003 Virus; Sequencing of the Envelope Gene and Neurovirulence Study

As an effort to give an insight into the molecular basis for the biological characteristic of CJ50003 strain, the 1500 nucleotide sequence encoding the envelope(E) gene which possesses major neutralizing epitopes were determined and compared with those of the parent vaccine strains, SA14-4-2(PDK), SA14-14-2(PHK) and an wild type SA14 virus (Aihira, S. et al. *Virus Genes*, 5:95–109, 1991; Ni, H. et al. *J Gen Virol*. 76:401–407, 1995; Ni, H. et al. *J Gen Virol*. 76:409–413, 1995; Nitayaphan, S. et al. *Virology* 177:541–542, 1990). CJ50003 virus (Vero, PS4) was used for sequence analysis. This revealed that the C-tenninal region (amino acid 280–500) shows complete conservation, while the N-terminal region (amino acid 1–279) shows sequence variation among the virus strains. Mutations in the N-terminal region are almost evenly distributed. Nucleotide sequence of the E protein gene of CJ50003 differed from SA14/CDC by 8 nucleotides and 7 amino acids whereas SA14-14-2(PDK) differed from SA14/CDC by 7 nucleotides and 5 amino acids. The results were summarized in Table 1.

The sequence of CJ50003 virus differed from the published sequence of SA14-14-2(PDK) virus at 5 positions: nucleotide changes at positions 1032, 1506, 1704 and 1769 resulted in 4 amino acid differences between SA14-14-2 (PDK) and CJ50003 viruses: nucleotide changes at position 989 did not result in amino acid substitution. Higher passages of CJ50003 virus, i.e. passage 15 and 30 revealed no additional nucleotide changes. There were, therefore 5 distinct nucleotide and 4 amino acid changes between CJ50003 and parent virus, SA14-14-2(PDK) and these changes were stable on passage of this virus in cell culture. The Lys residue at 243 in the SA14-14-2(PDK), which is uniquely different compared with other attenuated JE viruses were substituted with Glu residue in CJ50003.

CJ50003 sequence also differed from the published sequence of SA14-14-2(PHK) virus (Aihira, S. et al. *Virus Genes*, 5:95-109, 1991). The nucleotide difference at 1032 caused amino acid difference at position E19 but the change at nucleotide position 989 did not result in amino acid substitution. Nucleotides at 1506 and 1704 in CJ50003 virus were the same as those present in the SA14-14-2(PHK) at these positions while different from the SA14-14-2(PDK) at those positions. The pattern of substitutions through the N-terminal region of the CJ50003 and SA14-14-2(PHK) E gene is almost same except for amino acid substitution at E19.

SA14-14-2(PHK), SA14-14-2(PDK) and CJ50003 viruses have 4 identical amino acid substitutions compared with the sequence of the parent SA14 virus at position E107, E138, E176 and E279. Of those the amino acids at position E138 and E176 (Ni, H. et al. *J Gen Virol.* 76:409-413, 1995), which were known to contribute to attenuation were still conserved after Vero adaptation, suggesting that CJ50003 did not lose its attenuated character.

Vero cell substrate did not provide a neurovirulent phenotype to the SA14-14-2(PDK) and CJ50003 virus still has attenuated character.

TABLE 2

Intracerebral virulence of 4-week-old mice inoculated with Vero-passaged CJ50003 viruses. PS represents passage in PDK or Vero cell.

Virus	PFU Inoculum	log LD ₅₀ ml ⁻¹	LD ₅₀ /PFU ratio
SA14 (PDK, PS3)	2 × 10 (7)	6.5	0.17 ^a
SA14-14-2 (PDK, PS8)	1.3 × 10 (6)	<1.5 ^b	<0.00002 ^a
CJ50003 (Vero, PS6)	3.4 × 10 (7)	<1.5 ^b	<0.000001
CJ50003 (Vero, PS15)	3.2 × 10 (7)	<1.5 ^b	<0.000001
CJ50003 (Vero, PS30)	3.6 × 10 (7)	<1.5 ^b	<0.000001

^aKenneth H. Eckels et al (Vaccine 6: 513-518, 1988).

^b0/10 mice died after inoculation with undiluted virus.

The volume of inoculum for i.c. injection is 0.03 ml per mouse.

EXAMPLE 3

Virus Growth and Purification

The production seed was prepared in virus passage 5 in Vero cell [CJ50003 (Vero, PS5)] and stored in deep-freezer. Vero cells were grown in Eagle's minimal essential medium (EMEM, Gibco) containing 10% fetal bovine serum (FBS, Gibco). Roller bottle cultures of Vero cell monolayers were infected with production seed virus at an moi of 0.01 to 0.1 pfu per cell. After 2 hours of virus adsorption, the cultures were washed 3 times with PBS and fed with EMEM not containing serum and incubated at 35° C. In infected Vero

TABLE 1

Comparison of nucleotide and amino acid sequences among JE virus strains, SA14, SA14-14-2 (PHK), SA14-14-2 (PDK), and CJ50003.

Position	Nucleotide						Amino Acid					
	SA14/ USA	SA14/ CDC	SA14/ AP	SA14-14-2/ PHK	SA14-14-2/ PDK	CJ50003	SA14/ USA	SA14/ CDC	SA14/ AP	SA14-14-2/ PHK	SA14-14-2/ PDK	CJ50003
NT AA												
989 E4	G	G	G	U	U	G	Leu	Leu	Leu	Leu	Leu	Leu
1032 E19	A	A	A	A	A	G	Thr	Thr	Thr	Thr	Thr	Ala
1052 E25	G	A	A	A	A	A	Leu	Leu	Leu	Leu	Leu	Leu
1061 E28	U	U	U	C	C	C	Asp	Asp	Asp	Asp	Asp	Asp
1217 E80	C	C	U	U	C	C	Ala	Ala	Ala	Ala	Ala	Ala
1296 E107	C	C	C	U	U	U	Leu	Leu	Leu	Phe	Phe	Phe
1389 E138	G	G	G	A	A	A	Glu	Glu	Glu	Lys	Lys	Lys
1503 E176	A	A	A	G	G	G	Ile	Ile	Ile	Val	Val	Val
1506 E177	A	A	A	G	A	G	Thr	Thr	Thr	Ala	Thr	Ala
1704 E243	G	G	G	G	A	G	Glu	Glu	Glu	Glu	Lys	Glu
1708 E244	G	A	A	A	A	A	Glu	Gly	Gly	Gly	Gly	Gly
1769 E264	G	G	G	A	G	U	Gln	Gln	Gln	His	Gln	His
1813 E279	A	A	A	U	U	U	Lys	Lys	Lys	Met	Met	Met
1921 E315	C	U	C	U	U	U	Ala	Val	Ala	Val	Val	Val
1977 E334	C	C	U	C	C	C	Pro	Pro	Ser	Pro	Pro	Pro
2293 E439	A	G	A	G	G	G	Lys	Arg	Arg	Arg	Arg	Arg
2441 E488	G	A	G	A	A	A	Gly	Gly	Gly	Gly	Gly	Gly

AA: amino acid;

NT: nucleotide position strains.

CJ50003 and the parent SA14-14-2(PDK) were tested for their mice neurovirulence by intracerebral (i.c.) injection into the 4-week-old BALB/c mice. The results are shown in Table 2. The lethality for young adult mice is not significantly different between SA14-14-2(PDK) and CJ50003 viruses which is very low compared to that of wild type SA14 virus. Thus it seems to be that the introduction to the

cell cultures, virus reached titers of around 10⁷ to 10⁸ pfu/ml at 2 or 3 days post infection. While virus harvests were taken 4 times at 2 day intervals until 8 or 9 days post infection starting from 2 or 3 days post infection, virus titers were still maintained over 10⁷ pfu/ml with very weak CPE. But after 9 days post infection, the titers were under 10⁷ pfu/ml (FIG. 2). The pooled harvests were centrifuged at 8,000 rpm for 15

11

minutes and supernatants were filtered through a 0.45 μ m filter. Virus culture supernatants were concentrated by ultrafiltration (Ultrasette, Filtron, 100k) or precipitation with PEG. The virus precipitated by PEG was collected by centrifugation and suspended in PBS or STE (10 mM Tris pH 7.2, 1 mM EDTA, 150 mM NaCl) buffer. Retentate after ultrafiltration was concentrated to 250 ml and the cassette was washed with 100 ml of PBS. Virus concentrates were chilled in the ice for 2 hours after adding 0.5–2 mg/ml of protamine sulfate and the supernatants obtained by centrifuging at 10,000 rpm for 5 minutes. The concentrated viruses were purified by ultra-centrifugation on sucrose gradients. The ultracentrifugation was carried out at 38,000 g for 18 hours. Fractions were subjected to electrophoresis on polyacrylamide gels containing the detergent sodium dodecyl sulfate (SDS-PAGE). The nucleocapsid protein (C, 13,500 Da), membrane protein (M, 8,700 Da) and envelope protein (E, 53,000 Da) bands were seen in the SDS-PAGE (FIG. 3, panel A). Envelope antigens (E) were detected by Western blotting with mouse anti-JE virus monoclonal antibody (FIG. 3, panel C). Virus positive fractions, fraction Nos. 4 to 9, in which other protein bands except viral proteins were not apparent in silver stained gel (FIG. 3, panel B) were pooled, and assayed for protein concentration by Lowry method. Detailed results are shown from two purifications from infected Vero cultures either concentrated with tangential flow ultrafiltration or by PEG8000 precipitation (Tables 3 and 4). Purified virus was diluted with two volumes of PBS, added to the final 0.01% of Tween80, and filtered through a 0.22 μ m filter.

TABLE 3

Purification of JE virus by concentration with tangential flow ultrafiltration.						
Sample	Total Volume (ml)	Total pfu	% Yield (mg)	Total protein (mg)	% Yield (protein)	Specific Activity (pfu/mg)
Pooled culture supernatant	10,000	4.4×10^{11}	100	600	100	7.3×10^7
Filtrate concentrate	200	4.0×10^{11}	90	280	47	1.4×10^8
Sucrose gradient pool	500	3.8×10^{11}	86	42	7	9.0×10^9
0.22 μ filtration	50	2.4×10^{11}	55	23	3.8	1.0×10^{10}

TABLE 4

Purification of JE virus by concentration with PEG8000 precipitation.						
Sample	Total Volume (ml)	Total pfu	% Yield (pfu)	Total protein (mg)	% Yield (protein)	Specific Activity (psu/mg)
Pooled culture supernatant	10,000	4.4×10^{11}	100	600	100	7.3×10^7
PEG precipitate	200	2.7×10^{11}	61	40	6.7	6.8×10^9
Sucrose gradient pool	500	2.5×10^{11}	56	15	2.5	1.7×10^{10}
0.22 μ filtration	50	1.6×10^{11}	41	12	2	1.3×10^{10}

12

The virus preparations were compared for relative purity using specific activity measurements (i.e., pfu/mg protein). Virus purified from concentrate by ultrafiltration had about the same activity as virus purified from concentrate by PEG8000 precipitation. Also the purity of the pooled purified viruses was estimated by testing for Vero cell originated chromosomal DNA and protein. The results showed that contents of host cellular DNA and protein are as low as 2.5 pg and 2 ng per 5 μ g of purified JE virus respectively regardless of method of concentration, which demonstrated that both purification methods described above effectively removed other impurities from viral antigen. However in terms of protein yield of purified virus, the purification method using ultrafiltration is 2-fold better than the purification method involving PEG8000 precipitation.

EXAMPLE 4

Virus Inactivation

Purified virus was either directly used for preparation of live attenuated vaccine after dialysis with PBS or inactivated with formaldehyde for preparation of inactivated vaccine. Inactivation with 0.018% formaldehyde was carried out at 22° C. or 4° C. Samples were taken at regular intervals and assayed directly for infectious virus by plaque titration (FIG. 4). Samples which were negative by direct plaque assay were subjected to blind passage on Vero cell monolayers in order to amplify low levels of virus and then re-plaques. It was found that 4 days at 22° C. or 46 days at 4° C. were required for complete inactivation of infectivity (Tables 5 and 6). The antigenicity of the virus was monitored during inactivation by testing samples with the antigen spot blot assay and polyclonal antisera. By this assay there appeared to be no detectable losses in antigenicity after exposure to 0.018% formaldehyde for up to 10 days at 22° C. or 15 days at 4° C.

Inactivation with formaldehyde under these conditions was carried out for at least 7 days at 22° C. or 60 days at 4° C., giving a margin of safety. After inactivation, free formaldehyde in the samples was neutralized by the addition of 0.038% of sodium metabisulfite. Dialysis was carried out concurrently with PBS and then filtered through a 0.22 μ m filter.

TABLE 5

Formaldehyde inactivation of JE virus, CJS0003 at 4° C.			
Day	JE virus (-HCHO)	JE virus (+HCHO)	Amplification
0	3.2×10^8	3.2×10^8	+
0.5	2.6×10^8	3.2×10^6	+
1	1.52×10^8	7.9×10^5	+
1.5	2.4×10^8	4.8×10^5	+
2	2.8×10^8	6.4×10^4	+
3	2.6×10^8	4.3×10^3	+
4	1.9×10^8	1300	+
5	2.4×10^8	285	+
6	2.8×10^8	480	+
7	1.5×10^8	200	+
11	1.5×10^8	0	+
22	1.4×10^8	0	+
32	1.2×10^8	0	+
46	1.0×10^8	0	-
60	1.0×10^8	0	-

TABLE 6

Formaldehyde inactivation of JE virus at 22° C.			
Hour	JE virus (-HCHO)	JE virus (+HCHO)	Amplification
0	3.2×10^8	3.2×10^8	+
3	3.0×10^8	1.3×10^6	+
6	2.7×10^8	1.1×10^5	+
12	2.5×10^8	3.5×10^5	+
24	1.2×10^8	140	+
36	1.2×10^8	0	+
48	1.2×10^8	0	+
72	1.1×10^8	0	+
96	1.1×10^8	0	-
360	1.0×10^8	0	-

EXAMPLE 5

Immunogenicity of CJ50003 Purified, Inactivated Virus (PIV) and Live Attenuated Virus (LAV) in Mice

The immunogenicities of LAV and PIV were then tested in mice with previously commercialized Biken inactivated vaccine. Groups of 20 six-week-old BALB/c mice were immunized intraperitoneally (i.p.) with three kinds of immunogen. Immunization was done with two inoculations without an adjuvant at intervals of 2 weeks. Two weeks post second immunization sera were obtained from each group of mice, pooled and subsequently tested for the presence of neutralizing antibodies by PRNT method (Table 7). As shown in Table 7, there was no significant difference in neutralizing antibody titer between groups which received three kinds of immunogen.

TABLE 7

Induction of neutralizing antibodies in mice immunized with PIV or LAV			
Immunogen	Dose	Titers of neutralizing antibodies ^a	
PIV	5 μ g	1:320	
PIV	10 μ g	1:320	
LAV	5 μ g	1:320	
LAV	10 μ g	1:640	
Biken vaccine ^b	1 dose	1:320	

^aTiter of neutralizing antibody is defined as the reciprocal of serum dilution resulting in 50% reduction of mouse brain passaged Nakayama virus plaques.

^bBiken vaccine 1 dose contains 5 μ g of viral protein (TCA-precipitable) according to the manufacturer.

The Immunogenicity of PIV was further tested in mice. Adult, in bred mice were immunized with various dilutions of inactivated virus with or without an alum adjuvant. Groups of 20 six-week-old BALB/c mice were immunized subcutaneously with 500, 50, and 5 ng of PIV either in saline or saline with aluminum hydroxide (Rehydralgel). Mice received two inoculations spaced 3 weeks apart. Sera were pooled from each group of mice at 3 weeks post second immunization, and tested for the presence of neutralizing antibodies with mouse brain passaged Nakayama strain as neutralized virus (Table 8). PIV was better than Biken vaccine in all doses and adjuvant significantly improved the immune response of mice to 50 and 500 ng of PIV about 4 and 8 fold, respectively.

TABLE 8

Comparison of the titer of neutralizing antibody in mice immunized with PIV with or without alum hydroxide.

Immunogen	Dose	Titers of neutralizing antibodies ^a
PIV	500 ng	1:160
PIV	50 ng	1:40
PIV	5 ng	1:20
PIV + alum	500 ng	1:1280
PIV + alum	50 ng	1:160
PIV + alum	5 ng	1:20
Biken vaccine	1/10 dose	1:80
Biken vaccine	1/100 dose	1:10
Biken vaccine	1/1000 dose	1:10

^aTiter of neutralizing antibody is defined as the reciprocal of serum dilution resulting in 50% reduction of mouse brain passaged Nakayama virus plaques.

^bBiken vaccine 1 dose contains 5 μ g of viral protein (TCA-precipitable) according to the manufacturer.

The in vivo protective efficacy of PIV was then tested in BALB/c mice. For protection assays, groups of 10 three-week-old BALB/c mice were inoculated subcutaneously in the hindquarters with inactivated JE viruses in saline or saline with aluminum hydroxide (Rehydralgel). Age-matched controls were inoculated with PBS or non-specific antigens in alum. Mice were boosted with an equivalent dose three weeks later. The mice were challenged at 3 weeks post immunization by intracranial inoculation with 500 pfu of the mouse neurovirulent JE virus (Nakayama, mouse brain adapted) contained in 30 μ l of PBS. Challenged mice were monitored daily for morbidity and mortality for up to twenty-one days. As shown in Table 9, mice immunized with 50 ng of PIV showed 90% of protection. Furthermore, mice immunized with 50 and 5 ng of PIV mixed with alum showed 100% and 70% protection, respectively while 1/100 dose of Biken vaccine protected just 50% of immunized mice. In comparison, all mice in the control group became sick and died beginning at five to seven days post-challenge.

TABLE 9

Protection of vaccinated mice against challenge with Nakayama virus^a Immunogen

Immunogen	Dose	Survivors
Control ^b	N/A	0/10
PIV	500 ng	10/10
PIV	50 ng	9/10
PIV	5 ng	3/10
PIV + alum	500 ng	10/10
PIV + alum	50 ng	10/10
PIV + alum	5 ng	7/10
Biken vaccine ^c	1/10 dose	10/10
Biken vaccine	1/100 dose	5/10
Biken vaccine	1/1000 dose	3/10

^aMice immunized with 2 inoculations of test vaccines spaced 3 weeks apart, then challenged with 500 pfu of mouse-neurovirulent Nakayama virus.

^bAge-matched controls were inoculated with PBS or non-specific antigens in alum

^cBiken vaccine 1 dose contains 5 μ g of viral protein (TCA-precipitable) according to the manufacturer.

To investigate immunologic stability of CJ50003 virus over Vero cell passages, viruses with various passage numbers in Vero cell were independently purified and the immunogenicities were evaluated by the method as described in Table 8. As shown in Table 10, there was no remarkable difference in the ability to elicit neutralizing antibodies among vaccines prepared from the viruses with different virus passage numbers in Vero cell, indicating that CJ50003

15

virus is very stable over Vero cell passages in terms of immunogenicity.

TABLE 10

Vaccine potencies prepared with JE viruses with different virus passage numbers in Vero cells			
Immunogen ^a	Dose	Titers of neutralizing antibodies ^b	S.d. ^c
PIV-4ps	0.5 µg	1:150	20
PIV-6ps	0.5 µg	1:145	15
PIV-15ps	0.5 µg	1:130	28
PIV-20ps	0.5 µg	1:140	18
PIV-30ps	0.5 µg	1:160	13

^aImmunogen (PIV-Xps); purified inactivated vaccine prepared with CJ50003 virus of which passage number in Vero cells is X.

^bTiter of neutralizing antibody is defined as the reciprocal of serum dilution resulting in 50% reduction of mouse brain passaged Nakayama virus plaques and mean values of results from three separate experiments are taken. 50% endpoint is determined by Reed and Muench method.

^cStandard deviation

The results presented here suggest that both an inactivated JE virus vaccine and live attenuated vaccine using CJ50003 strain show promise. Relatively fast and efficient processes were developed for growing JE virus in Vero cell, concentrating and purifying them to a degree which may be suitable for human use and inactivating them without measurable loss in antigenicity. These preparations were found to be immunogenic and protective in mice.

16

What is claimed is:

1. An attenuated Japanese encephalitis virus adapted to Vero cell by passages on Vero cell wherein said virus has a multiplicity of more than 1×10^7 PFU/ml in Vero cells and LD_{50} /pfu for young adult mouse is less than 0.000001.

2. A Japanese encephalitis vaccine comprising the attenuated Japanese encephalitis virus according to claim 1.

3. The vaccine according to claim 2, which further comprises pharmaceutically acceptable additives.

4. The vaccine according to claim 2 wherein the virus is inactivated by an inactivating agent.

5. The vaccine according to claim 4, which further comprises pharmaceutically acceptable additives.

6. The vaccine according to claim 2 wherein the virus is live-attenuated JE virus untreated by an inactivating agent.

7. The vaccine according to claim 6, which further comprises pharmaceutically acceptable additives.

8. An attenuated Japanese encephalitis virus adapted to Vero cell by passages on Vero cell which is CJ50003.

9. A Japanese encephalitis vaccine comprising the attenuated Japanese encephalitis virus according to claim 8.

10. The vaccine according to claim 8, wherein the virus is inactivated by an inactivating agent.

11. The vaccine according to claim 8, wherein the virus is live-attenuated JE virus untreated by an inactivating agent.

12. The vaccine according to claim 9, which further comprises pharmaceutically acceptable additives.

* * * * *

Exhibit H

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,309,650 B1
DATED : October 30, 2001
INVENTOR(S) : Kim, Hyun Su et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title Page,

Item [75], please correct the name of the inventor(s) from:

**"Hyun Su Kim; Wang Don Yoo; Soo Ok Kim; Sung Hee Lee; Sang Bum Moon;
Sun Pyo Hong; Yong Cheol Shin; Yong Ju Chung; Kenneth H. Eckels; Bruce Innis;
Joseph R. Puniak; Leonard N. Binn; Ashok K. Srivastava; Doria R. Dubois"**
to read -- **Hyun Su Kim; Wang Don Yoo; Soo Ok Kim; Sung Hee Lee; Sang Bum
Moon; Sun Pyo Hong; Yong Cheol Shin; Yong Ju Chung; Kenneth H. Eckels;
Bruce Innis; Joseph R. Putnak; Leonard N. Binn; Ashok K. Srivastava; Doria R.
Dubois --**

Signed and Sealed this

Tenth Day of February, 2004

A handwritten signature in black ink, appearing to read "Jon W. Dudas". The signature is stylized with a large, looping initial "J" and a distinct "D" at the end.

JON W. DUDAS
Acting Director of the United States Patent and Trademark Office

Exhibit I



UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

Customer No 197

ISTMT

DATE PRINTED
04/06/2009

COMPUTER PATENT ANNUITIES
225 REINEKERS LANE
SUITE 400
ALEXANDRIA VA 22314

MAINTENANCE FEE STATEMENT

According to the records of the U.S. Patent and Trademark Office (USPTO), the maintenance fee and any necessary surcharge have been timely paid for the patent listed below. The "PYMT DATE" column indicates the payment date (i.e., the date the payment was filed).

The payment shown below is subject to actual collection. If the payment is refused or charged back by a financial institution, the payment will be void and the maintenance fee and any necessary surcharge unpaid.

Direct any questions about this statement to: Mail Stop M Correspondence, Director of the USPTO, P.O. Box 1450, Alexandria, VA 22313-1450.

PATENT NUMBER	FEE AMT	SUR CHARGE	PYMT DATE	U.S. APPLICATION NUMBER	PATENT ISSUE DATE	APPL. FILING DATE	PAYMENT YEAR	SMALL ENTITY?	ATTY DKT NUMBER
6,309,650	\$900.00	\$0.00	04/06/05	09/486,392	10/30/01	06/15/00	04	NO	012679-066



UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

Customer No 197

ISTMT

DATE PRINTED
04/06/2009

COMPUTER PATENT ANNUITIES
225 REINEKERS LANE
SUITE 400
ALEXANDRIA VA 22314

MAINTENANCE FEE STATEMENT

According to the records of the U.S. Patent and Trademark Office (USPTO), the maintenance fee and any necessary surcharge have been timely paid for the patent listed below. The "PYMT DATE" column indicates the payment date (i.e., the date the payment was filed).

The payment shown below is subject to actual collection. If the payment is refused or charged back by a financial institution, the payment will be void and the maintenance fee and any necessary surcharge unpaid.

Direct any questions about this statement to: Mail Stop M Correspondence, Director of the USPTO, P.O. Box 1450, Alexandria, VA 22313-1450.

PATENT NUMBER	FEE AMT	SUR CHARGE	PYMT DATE	U.S. APPLICATION NUMBER	PATENT ISSUE DATE	APPL. FILING DATE	PAYMENT YEAR	SMALL ENTITY?	ATTY DKT NUMBER
6,309,650	\$2,480.00	\$0.00	04/01/09	09/486,392	10/30/01	06/15/00	08	NO	CHEIL JEDANG CORP

Exhibit J

DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE FOOD AND DRUG ADMINISTRATION INVESTIGATIONAL NEW DRUG APPLICATION (IND) (TITLE 21, CODE OF FEDERAL REGULATIONS (CFR) PART 312)		Form Approved: OMB No. 0910-0014, Expiration Date: December 31, 1999 See OMB Statement on Reverse.
1. NAME OF SPONSOR Office of The Surgeon General, Department of the Army		2. DATE OF SUBMISSION <u>9 Sep 99</u>
3. ADDRESS (Number, Street, City, State and Zip Code) Commanding General, U.S. Army Medical Research and Materiel Command ATTN: MCMR-RCO-RA 504 Scott Street Fort Detrick, MD 21702-5012		4. TELEPHONE NUMBER (Include Area Code) 301-619-2165
5. NAME(S) OF DRUG (Include all available names: Trade, Generic, Chemical, Code) Japanese Encephalitis (JE) Purified Inactivated Virus Vaccine (Undiluted)		6. IND NUMBER (if previously assigned)
7. INDICATION(S) (Covered by this submission) Prophylaxis for Japanese encephalitis.		
8. PHASE(S) OF CLINICAL INVESTIGATION TO BE CONDUCTED: <input checked="" type="checkbox"/> PHASE 1 <input type="checkbox"/> PHASE 2 <input type="checkbox"/> PHASE 3 <input type="checkbox"/> OTHER _____ <div style="text-align: right;">(Specify)</div>		
9. LIST NUMBERS OF ALL INVESTIGATIONAL NEW DRUG APPLICATIONS (21 CFR Part 312), NEW DRUG OR ANTIBIOTIC APPLICATIONS (21 CFR Part 314), DRUG MASTER FILES (21 CFR Part 314.420), AND PRODUCT LICENSE APPLICATIONS (21 CFR Part 601) REFERRED TO IN THIS APPLICATION.		
10. IND submission should be consecutively numbered. The initial IND should be numbered "Serial number: 000." The next submission (e.g., amendment, report, or correspondence) should be numbered "Serial Number: 001." Subsequent submissions should be numbered consecutively in the order in which they are submitted.		SERIAL NUMBER <u>000</u>
11. THIS SUBMISSION CONTAINS THE FOLLOWING: (Check all that apply) <input checked="" type="checkbox"/> INITIAL INVESTIGATIONAL NEW DRUG APPLICATION (IND) <input type="checkbox"/> RESPONSE TO CLINICAL HOLD <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> PROTOCOL AMENDMENT(S): <input type="checkbox"/> NEW PROTOCOL <input type="checkbox"/> CHANGE IN PROTOCOL <input type="checkbox"/> NEW INVESTIGATOR </div> <div style="width: 30%;"> INFORMATION AMENDMENT(S): <input type="checkbox"/> CHEMISTRY/MICROBIOLOGY <input type="checkbox"/> PHARMACOLOGY/TOXICOLOGY <input type="checkbox"/> CLINICAL </div> <div style="width: 30%;"> IND SAFETY REPORT(S): <input type="checkbox"/> INITIAL WRITTEN REPORT <input type="checkbox"/> FOLLOW-UP TO A WRITTEN REPORT </div> </div> <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div style="width: 30%;"> <input type="checkbox"/> RESPONSE TO FDA REQUEST FOR INFORMATION <input type="checkbox"/> REQUEST FOR REINSTATEMENT OF IND THAT IS WITHDRAWN, INACTIVATED, TERMINATED OR DISCONTINUED </div> <div style="width: 30%;"> <input type="checkbox"/> ANNUAL REPORT <input type="checkbox"/> OTHER _____ </div> <div style="width: 30%;"> <input type="checkbox"/> GENERAL CORRESPONDENCE <div style="text-align: right;">(Specify)</div> </div> </div>		
CHECK ONLY IF APPLICABLE		
JUSTIFICATION STATEMENT MUST BE SUBMITTED WITH APPLICATION FOR ANY CHECKED BELOW. REFER TO THE CITED CFR SECTION FOR FURTHER INFORMATION. <input type="checkbox"/> TREATMENT IND 21 CFR 312.35(b) <input type="checkbox"/> TREATMENT PROTOCOL 21 CFR 312.35(a) <input type="checkbox"/> CHARGE REQUEST/NOTIFICATION 21 CFR 312.7(d)		
FOR FDA USE ONLY		
COR/DB/IND/OGD RECEIPT STAMP	DOR RECEIPT STAMP	DIVISION ASSIGNMENT: IND NUMBER ASSIGNED:

Exhibit K



DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
Rockville MD 20857

Intercell USA, Inc.
Attention: Paul J. Wilson
179 Castles Gate Drive
Mooresville, NC 28117

December 28, 2007

Dear Mr. Wilson:

We have received your biologics license application (BLA) submitted under section 351 of the Public Health Service Act for the following biological product:

Our Submission Tracking Number (STN): BL 125280/0

Biological Product: Japanese Encephalitis Vaccine

Indication: For the Active immunization against Japanese Encephalitis Virus.

Date of Supplement: December 18, 2007

Date of Receipt: December 20, 2007

First Action Due Date: October 19, 2008

US License Number: 1790

We will notify you within 60 days of the receipt date if the application is sufficiently complete to permit a substantive review.

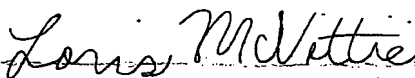
Please submit all future correspondence, supporting data, or labeling relating to this application in triplicate, citing the above STN number. Send all correspondence to the following address:

Norman Baylor, Ph.D., HFM-475
Center for Biologics Evaluation and Research
Food and Drug Administration
Suite 200N
1401 Rockville Pike
Rockville, MD 20852-1448

Page 2 – STN BL 125280/0

If you have any questions, please contact Dr. Richard Daemer or Ms. Daryll Miller, Regulatory Project Managers, at (301) 827-3070.

Sincerely yours,

A handwritten signature in cursive script, reading "Loris D. McVittie", is written over a horizontal line.

Loris D. McVittie, Ph.D.
Chief
Viral Vaccine Branch
Division of Vaccines and
Related Products Applications
Office of Vaccines
Research and Review
Center for Biologics
Evaluation and Research

Exhibit L

**IND 8589 Japanese Encephalitis Virus (SA 14-14-2; Vero cells) Formalin
Inactivated, Purified Vaccine**

Serial number	Contents	Date of submission
0000	Initial IND submission, clinical phase 1 study: HSRRB Log No. A-9210	9 September 1999
0001	Protocol amendment to clinical phase 1 study: HSRRB Log No. A-9210; safety of booster dose	25 July 2000
0002	Annual report 10 Sept 1999-9 Sept 2000	20 September 2000
0003	Clinical study protocol HSRRB Log No. A-10160	5 January 2001
0005	Protocol amendment to clinical phase 1 study: HSRRB Log No. A-10160; additional associate investigator: Arthur Lyons	8 May 2001
0006	Answer to FDA letter from 12 March 2001 ; Vero cells for vaccine production	11 July 2001
0007	Annual report 10 September 2000-9 September 2001, Investigator's Brochure update	25 October 2001
0008	Protocol amendment to clinical phase 1 study: HSRRB Log No. A-10160; additional 2 clinical laboratories	8 January 2002
0009	Meeting request end of phase 2 Meeting request for type C	27 March 2002
0010	Briefing package for a type C teleconference	3 May 2002
0011	Meeting minutes to the teleconference on 10 June 2002 with FDA by WRAIR	12 July 2002
0012	Annual report 10 Sept 2001 – 9 September 2002, Investigator's Brochure update	18 September 2002
0013	IND transfer WRAIR – VaccGen	20 September 2002
0014	Annual report 10 September 2002 – 9 September 2003	7 November 2003
0004	CMC update	30 January 2001
0015/1	Final study report phase 1 (HSRRB Log No. A- 9210)	21 June 2004
0015/2	Final study report phase 2 (HSRRB Log No. A- 10160)	21 June 2004
0016	IND transfer letter VaccGen Int. LLC to Intercell USA, Inc.	24 June 2004
0017	Meeting request for a type B end of phase 2	30 June 2004
0018	IND acceptance letter by IND Intercell USA, Inc.	12 July 2004
0019	Technical Briefing Package for the type B end of phase 2 meeting scheduled for September 28	25 August 2004
0020	Meeting minutes of End of phase 2 meeting	11 October 2004
0021	Annual report Sept 10 2003 –Sept 9, 2004 Investigator's Brochure update	5 November 2004
0022	Comments to 25 Oct 2004 dated official FDA meeting minutes - CMC part - of the meeting on Sept 28, 2004	6 December 2004
0023	Minutes of the Telephone conference on phase 3 clinical aspects on 16 November 2004	7 December 2004
0024	Protocol for the IC51-301 Phase 3 clinical trial	7 December 2004

**IND 8589 Japanese Encephalitis Virus (SA 14-14-2; Vero cells) Formalin
Inactivated, Purified Vaccine**

0025	Protocol for IC51-302 Phase 3 clinical trial (version 1.0) 050311 Intercell-FDA Stat Comments (3-09-05)	4 January 2005 11 March 2005
0026	Updated protocol for IC51-301 (version 3.1) Phase 3 clinical trial	4 January 2005
0027	Protocol for IC51-303 Phase 3 clinical trial (version 1.0)	4 January 2005
0028	Response to FDA questions from 27 January 2005 regarding IC51-301-302 -304 -305	23 March 2005
0029	Protocol for validating the JEV inactivation step	12 April 2005
0030	Responses to FDA's statistical comments regarding to Protocols IC51-302 and IC51-303	26 April 2005
0031	Questions for CBER regarding the filling scale and stability data starting the phase 3 trials	02 June 2005
0032	Protocol for plaque reduction neutralization test (PRNT) and data from JEV immune sera tests received from WRAIR	13 June 2005
0033	Request for teleconference with FDA to discuss start of Phase 3 clinical trials with IC51 (JE-PIV) in September 2005. It will be demonstrated that IC51 (JE-PIV) produced at WRAIR is comparable with material produced at IC's manufacturing site in Scotland.	22 June 2005
0034	Protocol for the concomitant vaccine clinical trial IC51-308 which is designed to study safety and immunogenicity of IC51 (JE-PIV) co-administered with Hepatitis A vaccine. IC51-308 study will be started in September or October 2005. FDA comments on concomitant trial Sender: FDA, Laraine Henschel Recipient: Intercell USA, Paul Wilson; Intercell AG	7 July 2005 17 August 2005
0035	Protocol for conducting the "Developmental Toxicity Study in Rats". Request for FDA comments. FDA answer to IC request for comments on Comparability Report and FDA response to the protocol for the developmental toxicology study (#35).	8 July 2005 29 July 2005
0036	FDA is notified about Barbara Veprek who will be responsible for monitoring the conduct and progress of Phase 3 clinical trials from now on.	22 July 2005
0037	IC51 Interim Comparability Report	5 August 2005
0038	Addendum to the Interim Comparability Report submitted on 5 th August 2005	2 September 2005

**IND 8589 Japanese Encephalitis Virus (SA 14-14-2; Vero cells) Formalin
Inactivated, Purified Vaccine**

0039	Certificate of Analysis for Phase 3 Clinical Trial Material (Lot Number ICB05/500) and placebo.	15 September 2005
0040	Approval forms to start IC51 phase 3 and demonstration of JE-PIV non-inferiority to JE VAX® (see paper & electronic version).	3 October 2005
0041	Annual Report for IND 8589 covering the reporting period from 10 th September 2004 to 9 th September 2005, containing a summary of significant manufacturing changes and an updated investigational plan for the coming year.	7 November 2005
0042	Submission 0042 contains the 1572 forms, investigators' credentials and financial disclosure forms for sites in study IC51-302 that have begun enrolling subjects. Enclosed are also investigators credentials for sub investigators of IC51-301. (Due to the volume of 781 pages find submission 0042 as CD and as copy Binder 10A/I and 10A/II in the archive.)	21 November 2005
0043	IND 0043 is a follow-up to IND 0040 and IND 0042 to provide updated investigator information on the studies IC51-301 and IC51-302. It contains investigators credentials, financial disclosure forms and updated 1572 study site forms. (see paper & electronic version)	22 December 2005
0044	Request for Type C meeting to discuss questions related to the clinical and eventual commercial manufacture for IC51 JE-PIV and the regulatory timelines regarding BLA submission in late 2006.	11 January 2006
0045	IND 0045 is a follow up to IND 0040, 0042 and 0043. It provides updated investigator information for the studies IC51-301 and IC51-302 containing investigators credentials, financial disclosure forms and updated 1572 study site forms – additionally for new sites in IC51-302 where patient enrollment has started. (Original CBR-CD containing IND 0045 & IND 0046 is stored within IND 0045).	17 January 2006
0046	IND 0046 contains investigator information for principal investigators and sub-investigators participating in Studies IC51-303-304-305.	27 January 2006
0047	Protocol Amendment: New Investigators: contains investigator information for principal investigators and sub-investigators participating in Phase 3 clinical studies, IC51-303 and IC51-308.	20 February 2006
0048	Revised Clinical/Regulatory Plan Timeline changes for submission of clinical data regarding IC51 (JE-PIV) licensure from	23 February 2006

**IND 8589 Japanese Encephalitis Virus (SA 14-14-2; Vero cells) Formalin
Inactivated, Purified Vaccine**

	November 2006 to March 2007.	
0049	Type C Meeting Briefing Package for Type C meeting on 21 March 2006.	27 February 2006
0050	Phase 3 clinical protocol for IC51-309 in order to conduct a clinical batch comparison of IC51 (JE-PIV).	09 March 2006
0051	Investigational Brochure update, Version 4.0 for Japanese encephalitis vaccine; IC51 (JE-PIV). This version captures information about the product till February 28, 2006.	17 March 2006
0052	Current draft of Statistical Analysis Plan for phase 3 clinical study IC51-301 as follow-up for the Type C meeting on 21 March 2006.	23 March 2006
0053	Intercell summary of Type C Meeting held on March 21 March 2006 & Type C Meeting Minutes/21 March 2006 by Laraine Henschel (dated 20 April 2006)	6 April 2006
0054	Request for pre BLA Meeting for IC51 JE-PIV Between July 17 and 26, 2006	9 May 2006
0055	Intercell's point-by-point response to FDA's comments on Statistical Analysis Plan.	16 May 2006
0056	Intercell's postponement of face-to-face pre-BLA meeting in early September 2006 and withdrawal of pre-BLA meeting request submitted under Serial Number 0054, dated May, 9 2006.	19 May 2006
0057	Intercell Responses to Statistical Comments received with FDA Meeting Minutes dated March, 21 2006 Type C Meeting	1 June 2006
0058	Request for Type C Meeting for discussion of Intercell's facility for manufacturing IC51 (JE-PIV).	12 June 2006
0059	Final draft of Intercell's Statistical Analysis Plan (SAP) for IC51-302	23 June 2006
0060	Request for face-to face pre-BLA meeting to be scheduled on September 6 or 7, 2006.	30 June 2006
0061	Background information package for the Type C meeting between Intercell and FDA scheduled for 16 August 2006. Meeting purpose is to discuss questions to Intercell's commercial manufacturing facility (see paper & electronic version).	14 July 2006
0062	Modified protocol for the lot-to-lot consistency study IC51-309 planned for initiation in September 2006.	20 July 2006
0063	Response to FDA Facility Questions IND 0063 contains Intercell's responses to the FDA questions regarding the briefing package (serial 0061 dated July, 14 2006) for upcoming Type C telephone conference, scheduled August 16 2006 to discuss	11 August 2006

**IND 8589 Japanese Encephalitis Virus (SA 14-14-2; Vero cells) Formalin
Inactivated, Purified Vaccine**

	Intercell's commercial manufacturing facility for IC51 (JE-PIV).	
0064	Final Comparability Report Final Comparability Report for Intercell's phase 3 clinical supplies for IC51 JE-PIV. Report is follow-up to Serial 037 submitted August 05, 2005.	18 August 2006
0065	Pre-BLA Meeting Information Package Technical Briefing package for pre-BLA meeting on September, 19 2006 (see submission enclosed as electronic file)	21 August 2006
0066	Type C Facility Meeting Summary Intercell's summary of the Type C Meeting held by telephone conference on August, 16 2006 regarding design and operation of Intercell's commercial manufacturing facilities.	07 September 2006
0067	Filing and Review Periods Projected timelines for the manufacture and testing of process conformance batches of IC51 (JE-PIV).	13 September 2006
0068	IC51-303 Statistical Analysis Plan I Intercell's request for FDA comments on IC51-303 Statistical Analysis Plan Draft 1.0 dated September 13, 2006	25 September 2006
0069	Protocol Amendment – New Investigator Information on new principal investigators and sub-investigators in Intercell's phase 3 clinical studies IC51-301, IC51-302, IC51-303 and IC51-308. (Electronic version is stored in the archive)	26 September 2006
0070	Final Clinical Study Report for Study IC51-301	11 October 2006
0071	Pre BLA Meeting Minutes	13 October 2006
0072	Annual Report for IND 8589 covering the reporting period from September 10, 2005 to September 9, 2006.	7 November 2006
0073	Intercell informed the FDA Division of Vaccines and Related Products Applications that it has submitted a request for orphan drug designation for its Japanese encephalitis vaccine on November 08, 2006.	8 November 2006
0074	Submission of a 15-day safety report for a serious adverse event which occurred in the study IC51-302.. A female subject, enrolled in the study, discovered on 31 January 2006 that she was pregnant. The baby who was delivered on 20 September 2006 was found to have a congenital anomaly consisting of unilateral syndactyly of the second and the third toe. In addition, an enlarged renal pelvis with suspicion of vesico-urethral reflux was revealed Intercell confirmed to forward the initial safety report to the appropriate regulatory authorities	16 November 2006
0075	Submission 0075 contains FDA Forms 1572 and Curriculum Vitae relating to the participation of investigators in Intercell's Phase 3 clinical study IC51-309, additionally FDA Form 1572 and Curriculum Vitae regarding change in Principal	17 November 2006

**IND 8589 Japanese Encephalitis Virus (SA 14-14-2; Vero cells) Formalin
Inactivated, Purified Vaccine**

	Investigator at a clinical site in study IC51-303.	
0076	Intercell's questions and comments regarding the SAP for the study IC51-303, and comments related to future BLA submissions.	28 November 2006
0077	Final Clinical Study Report for study IC51-302 "Safety and Tolerability of the Japanese Encephalitis Vaccine IC51 (JE-PIV). Double-Blind, Randomized, Placebo Controlled Phase 3 Study".	30 November 2006
0078	Statistical Analysis Plan (SAP) for the Phase 3 lot-to-lot consistency study IC51-309.	10 January 2007
0079	SAP for evaluation of safety at 6 months post vaccination for IC51, Intercell's Japanese encephalitis virus vaccine. Datas from the following studies are included: IC51-301, IC51-302, IC51-303, IC51-304, IC51-305, IC51-308 and IC51-309.	26 January 2007
0080	Section 14.1 / Tables for Clinical Study Report IC51-301 (supplementary to Serial 070, submitted October 11, 2007)	29 January 2007
0081	Forms 1572 and CVs documenting investigator change in two sites of phase 3 clinical trial IC51-302 and IC51-303, addition of a new sub-investigator and roll-over of Dr. Lyon's site from IC51-302 to IC51-303.	2 February 2007
0082	Follow-up IND Safety Report for the Serious Adverse Event occurred in study IC51-302 and reported to the Agency on November 16, 2006 as Serial 0074.	15 February 2007
0083	CMC update for IC51 (JE-PIV)	9 March 2007
0084	Acknowledgement of comments on Statistical Analysis Plan IC51-309	4 April 2007
0085	Clinical Study Report IC51-309 (signed 26 April 2007) excluding Tables, Figures and Listings	30 April 2007
0086	Request for Guidance regarding Clinical Dataset	4 May 2007
0087	Plaque Reduction Neutralization Test Assay Validation Report	7 May 2007
0088	Comparability Protocol	9 May 2007
0089	Type C Meeting Request	10 May 2007
0090	Investigator's Brochure update for IC51	21 May 2007
0091	Final Report for six months safety analysis across the Phase 3 studies IC51-301, -302, -303, 304, 305 and-308 including 2922 patients who received at least one vaccination with IC51.	11 June 2007
0092	Briefing Package for Type C teleconference scheduled for 10:00- 11:30 am EDT on July, 16 2007 to discuss clinical, manufacturing and regulatory issues.	22 June 2007
0093	Intercell's response to FDA's comments on Clinical Datasets.	28 June 2007
0094	Proposed Brand Name IXIARO	20 July 2007
0095	Type C Telephone Conference Meeting Minutes held on 16 th July 2007 to discuss questions related to CMC information, clinical data from study IC51-309 and regulatory strategy regarding BLA submission for	24 July 2007

**IND 8589 Japanese Encephalitis Virus (SA 14-14-2; Vero cells) Formalin
Inactivated, Purified Vaccine**

	IC51 in fourth quarter 2007.	
0096	Additional Information on Proposed Proprietary Name/Brand Name	10 September 2007
0097	Summary Report for Investigation of Failed Clinical Lots	11 October 2007
0098	Annual Report covering reporting period from 10 th September 2006 to 9 th September 2007	9 November 2007
0099	eCTD test submission	26. November 2007
0100	Clinical Study Report Phase 2 Study IC51-221 CD in Archive only	16. July 2008
0101	Investigator's Brochure Version 6.0	07. November 2008
0102	Annual Report 10 September 2007 – 09. September 2008	10. November 2008

BLA 125280 IXIARO®
Japanese Encephalitis Virus (strain SA 14-14-2) Formalin Inactivated, Purified
Vaccine

Serial Number	Content	Submission Date
0000	BLA	20 Dec 2007
0001	Draft package insert for IXIARO in Microsoft Word format Pharmacovigilance Plan.	14 February 2008
0002	Response to information requested by the Agency in a letter dated 3 March 08 <u>Updated sections</u> <ul style="list-style-type: none"> • Section 1.9.1: Request for Waiver of Pediatric Studies • Section 3.2.S.2.1: MHRA Manufacturing Authorization and GMP Certificate • Section 3.2.P.3.1: MHRA Manufacturing Authorization and GMP Certificate • Section 4.2.3.5.3.7: Summary of Historical Background Data in Developmental Toxicity and Pre and Post Natal Studies • Section 5.3.5.1.9: Clinical Study Report for Study IC51-310 • Section 5.3.5.3: Tabular listing of all subjects in all studies • Section 5.4: Reference on communication with the Agency in terms of "Data Tabulation Datasets". • Section 3.2.R.1 has been updated to include some additional information we have received from the filling site (Vetter) 	4 April 2008
0003	Responses to the information requested by the Agency during a teleconference between CBER and Intercell on April 28, 2008. The answers are provided in section 1.11.3 no other section is updated	2 May 2008
0004	Response to information requested by the Agency in a fax dated 1 May 08 See section 1.11.1 Clinical Study protocol and amendment for study IC51-310, the Statistical analysis plan for IC51-310	16 May 2008
0005	<ul style="list-style-type: none"> • Updated Section 1.16, Risk Management Plans • Updated Section 1.16, Enhanced Post marketing Surveillance Plan • Section 1.11.4, Request for Review of Proprietary name • Section 4.2.3.5.3 Individual data from animals showing no abnormalities (Reproductive toxicology study) 	06. June 2008
0006	<ul style="list-style-type: none"> • Update Section 3.2.P.8.3, Stability Update • New Section 5.3.5.1.10, IC51-310 Study Report, including appendices 	23. June 2008
0007	<ul style="list-style-type: none"> • Section 5.3.5.1.10, IC51-310 Clinical Study Datasets • Section 3.2 R, Lot release SOPs 	01. July 2008
0008	<ul style="list-style-type: none"> • Responses to questions from the Agency in an email from Daryll Miller dated July 25, 2008. • An additional histopathological analysis done on the animals involved in the reproductive toxicology study. This analysis is 	11. August 2008

BLA 125280 IXIARO®
Japanese Encephalitis Virus (strain SA 14-14-2) Formalin Inactivated, Purified Vaccine

	presented in Section 4.2.3.5.3.9, Appendix 8. This analysis was suggested by the EMEA as a result of the review of our license application	
0009	The Amendment provides Intercell's responses to comments raised by the Agency in an email from Dr. Daemer dated August 14, 2008 related to the Post-marketing Safety Evaluation and Pharmacovigilance Plans for IXIARO®.	08. September 2008
0010	<p>Responses to FDA 483 (initial response submitted in June, the package submitted in August, and the Stedim Flexboy Bag Integrity Report SD117448 rev 00), provided in Module 1.</p> <ul style="list-style-type: none"> • Additional information on the dye ingress test (integrity of the final vaccine container closure system), calculation of potential dye flow, included in 3.2.P.2.4-Appendix 4a. • Draft release protocol, included in 3.2.R.3.P-Appendix 8. <p>The following sections of Module 3 have been updated in this submission due to the 483 observations and the information request from FDA dated July 25, 2008, which was already answered through Amendment 0008.</p>	25. September 2008
0011	<ul style="list-style-type: none"> • Intercell's complete response to the Complete Response (CR) Letter issued by FDA on September 26, 2008 (attached to this letter). Intercell's response has been included in Section 1.11.4 of the BLA. • An updated version of the Post-marketing Safety Evaluation Plan (Section 1.16) based on guidance from the Agency. 	01. October 2008
0012	<ul style="list-style-type: none"> • Waiver to submit post-marketing safety updates in ICH format and in 6-month intervals. This request has been placed in Section 1.12 of the BLA. Please note that this request is addressed specifically to Dr. Robert Ball of the Office of Biostatistics & Epidemiology, CBER. • Updated draft Lot Release Protocol submitted in 3.2.R.3.P-Appendix 8. • Proposed specification for Protamine sulphate content in Pre-dilution Sucrose Gradient Pool (for each run). 	24. October 2008
0013	Priority Review Voucher according to Section 524 of the Federal FD&C Act. This application is addressed to the Office of the Secretary, HHS and is placed in Section 1.7.1	03. November 2008
0014	<p>Section 1.11.3 Response to questions regarding PI</p> <p>Section 1.14.1.3 Prescribing Information</p> <p>Section 1.11.4 Request for Final Review of Proprietary Name</p> <p>Section 3.2.R.3.P Appendix 8 Lot Release Protocol</p>	17. November 2008
0015	<p>Section 1.11.3 Response to Questions</p> <p>Section 5.3.5.1 IC51-302 Case Report</p> <p>Section 5.3.5.1 IC51-303 Case Report</p>	01. December 2008
0016	<p>Section 3.2.R.3.P Appendix 8, Final Lot Release Protocol</p> <p>Section 5.3.5.1, Subject records 2103-009 (alleged dermatomyositis).</p>	15. December 2008

BLA 125280 IXIARO®
Japanese Encephalitis Virus (strain SA 14-14-2) Formalin Inactivated, Purified Vaccine

0017	Section 1.14.1.1 Syringe Labels Section 1.14.1.1 Packaging Box (private sector) Section 1.14.1.1 Packaging Box (military sector)	16. December 2008
0018	<ul style="list-style-type: none"> • Updated Labelling (submitted to CBER by email Dec. 2008) • Meeting Minutes (Telecon regarding commitments; submitted to CBER by email Dec. 2008) • Synopsis of Post-Marketing Study in Elderly (submitted to CBER by email Dec. 2008) 	12. January 2009
0019	Lot release protocols of the 3 consistency batches	12. January 2009
0020	Response to e-mail sent on 05. January 2009 regarding Agency's feedback on the post-marketing studies.	12. January 2009
0021	Updated according to guidance provided by Agency on 09. Jan.2009 Section 1.14.1.1 Syringe Label Section 1.14.1.1 Packaging Box (private sector) Section 1.14.1.1 Packaging Box (military sector)	19. January 2009
0022	This Amendment contains the updated paediatric development plan, including synopses of the proposed post-marketing studies in the paediatric population in response to the Agency's request during a teleconference on January 16, 2009.	21. January 2009
0023	Section 1.14 Draft Prescribing Information (commercial) Section 1.14 Draft Prescribing Information (Military)	27. January 2009
0024	This Amendment contains Lot Release Protocols for the two (2) lots that are currently being tested by CBER and which Intercell intends to release for commercial use post-BLA approval.	30. January 2009
0025	Section 1.12.14 Environmental Assessment	12. February 2009
0026	Section 3.2.S.2.3 Control of Materials Section 3.2.S.2.3 Appendix 6 Testing of End of Production Cell Bank	16. February 2009
0027	<ol style="list-style-type: none"> 1. Sample submission and shipping documentation (attached to this cover letter) 2. Lot Release Protocols for sample lots were submitted under Amendment 0019, section 3.2.R.3.P Appendix 9. 	26. February 2009
0028	Timelines for Post-Licensure Clinical Trials	13. March 2009
0029	Information regarding teleconference of 6.March 2009	25. March 2009

Exhibit M



**Development Summary of IC51,
A Japanese encephalitis purified inactivated
vaccine**

Intercell AG
Vienna Biocenter 3
A-1030 Vienna
Austria

Table of Content

<i>Manufacturing</i>	3
<i>Non-clinical Studies</i>	3
Pharmacology	3
Non-clinical and toxicology studies	3
Safety study in animals	3
IC51 - Pre and post natal developmental toxicity study in rats	3
<i>Clinical</i>	3
<i>Regulatory</i>	3
<i>References</i>	3

List of Tables

Table 1:	Antigenic potency of different batches of IC51 and JE-VAX [®] vaccines in mice	3
Table 2:	IC51 and JE-VAX [®] vaccine protective efficacy in mice.....	3

Introduction

IC51 is a purified and inactivated Japanese encephalitis virus vaccine derived from the attenuated virus strain SA₁₄-14-2. The virus is grown in Vero cells, purified, inactivated with formaldehyde, and then adsorbed on aluminium hydroxide. The final vaccine is supplied in a pre-filled syringe. Each 0.5-mL dose of IC51 contains 6 mcg of the inactivated Japanese encephalitis virus (attenuated strain SA₁₄-14-2 grown in Vero cells) adsorbed on aluminium hydroxide, hydrated (approximately 2.5 mg Al).

IC51 is indicated for active immunization against Japanese encephalitis (JE) for persons aged eighteen years and older. Two immunizations (each containing 6 mcg inactivated attenuated JEV strain SA₁₄-14-2) given four weeks apart are required to achieve optimal protection against JE. IC51 (JE-PIV) is to be injected intramuscularly in the deltoid region and should be administered by an authorized health care professional.

Initial clinical evaluation of the IC51 vaccine (through Phase 2) was conducted by the Walter Reed Army Institute of Research (WRAIR) which is part of the US Army. Intercell sublicensed the vaccine in 2003 and proceeded with the manufacturing and clinical development based on discussions with both EMEA and FDA.

In the USA, IC51 is the subject of an active IND (8589), which has been transferred from VaccGen to Intercell.

In May 2005, Intercell received Scientific Advice from the CHMP (EMA/H/SA/558/1/2005/III), regarding the proposed development program for IC51. The Phase 3 program of IC51 was started after discussion of the development programs with FDA (under IND) and EMA (under Scientific Advice).

Intercell submitted license applications in Australia, US, Europe (through EMA centralized procedure), Canada and Switzerland. The product has so far been approved in Australia, USA and EU with decisions from Canada and Switzerland awaited.

Development of the product

As mentioned earlier, IC51 was originally developed by WRAIR, which is part of the US Department of the Army, and VaccGen International LLC, a US-based company.

The original virus strain JEV SA₁₄-14-2 was developed in China. It was imported to WRAIR, USA and passaged in primary canine kidney (PDK) cells (Eckels *et al* 1988). This strain was then adapted to growth in Vero cells. Analysis was done to show equivalence in terms of morphology, antigenicity for the newly adapted strain of virus. This new adapted strain was used to produce the vaccine (Srivastava *et al* 2001) and to conduct trials in humans.

Phase 1, 2 and 3 clinical trials showed good profile for safety and immunogenicity, details are summarized in the clinical section.

Manufacturing

The manufacturing of IC51 is similar in broad terms with other viral vaccines produced in Vero cells. The details are mentioned in the 'commercial' part of the manufacturing. Based on the experience gained in the earlier Phase 1 and 2 manufacturing and scale-up, a commercial manufacturing process was developed.

Phase 1: Lot 0475, was prepared from a total of 10 L of virus harvest that was pooled, clarified, filtered and ultrafiltered. The concentrated virus was treated with protamine sulfate to remove the Vero cell DNA. Then the virus was purified by zonal centrifugation in sucrose gradients. The gradient fractions containing hemagglutinating virus were formalin inactivated for 10 days at 22°C, then neutralized with sodium metabisulphite and stored at 4°C. In order to remove the residual formaldehyde and sodium metabisulphite, the JE Virus (SA₁₄-14-2) Purified Inactivated Bulk Vaccine, lot 0475, was dialyzed against Phosphate Buffered Saline (PBS) two times at 4°C between 19 - 23 hours each time. This step was removed from the process in subsequent stages of development. Post dialysis, the JE PIV Bulk Vaccine was sterile filtered and tested. A volume of 15.0 ml of Rehydralgel aluminum hydroxide was aseptically added to 300 ml of the IC51 dialyzed Bulk Vaccine, lot 0574. A total of 3.15 ml of 1 % (w/v) thimerosal in PBS was added to the formulated bulk. 294.15 ml of the formulated bulk was (lot 0574), was filled at 4 ml ± 5% to give a total of 70 vials.

Phase 2: For the Clinical Trial Material required for Phase 2, minor changes in the production scheme were introduced, such as a final bulk vaccine increase from 10L to 20L, the elimination of final dialysis of the neutralized bulk vaccine due to major losses of immunogenicity at this step and the elimination of the addition of thiomersal. All other process steps remained unchanged.

The process ended with JE PIV Bulk Lot 0664 that was filled into final containers as Lot 0737.

Phase 3: Phase 3 material was produced in the scale of 200 roller bottles at Intercell's facility in Livingston, U.K. The manufacturing process was also similar to the earlier processes except minor changes due to process optimization and scale-up that were addressed through the execution of a comparability protocol and comparability report.

Commercial

The commercial process is similar to the Phase 3 process with some modifications introduced to further optimize the manufacturing process (e.g. new clean room suite in the Drug Substance manufacture, new filling facility for manufacture of Final Vaccine Lot, change of aluminium hydroxide supplier)

Vero (African Green Monkey kidney) cells are used to propagate Japanese Encephalitis virus (JE) (strain SA₁₄₋₁₄₋₂). The working viral seed bank has been produced by Intercell Biomedical Limited Scotland. IC51 (JE-PIV) bulk vaccine lot is manufactured by infecting Vero cells grown in roller bottles. After inoculation, the virus is harvested on days 3, 5, 7 and 9, post infection. These virus-containing harvests are filtered and concentrated. The concentrated virus is treated with protamine sulphate to remove Vero cell DNA/protein, and is subsequently purified by sucrose density gradient centrifugation. The purified live virus is inactivated with formaldehyde which is then neutralized with sodium metabisulphite. Following neutralization, the inactivated viral solution is sterile filtered to 0.22µ, adsorbed onto aluminum hydroxide (Alhydrogel, Brenntag Biosector) and stored in Flexboy bags for shipment to the contract filling facility where it is filled into single use, nominal 1.25mL borosilicate type 1 glass syringes.

Manufacture of Drug Substance and Final Bulk Vaccine is performed at Intercell's facility in the U.K (approved by MHRA for the manufacture of IMP and manufacture of commercial material). Three manufacturing consistency lots of IC51 (JE-PIV) of Final Bulk Vaccine were manufactured in the Livingston facility.

The filling of Final Bulk Vaccine is carried out at Vetter Pharma-Fertigung GmbH & Co. KG, 88212 Ravensburg, Germany. The container closure system has remained unchanged from the consistency lots.

A comparability exercise was performed. The comparability protocol covered all changes implemented between Phase 3 clinical lots manufacture and commercial manufacture, including change of clean room suite, of filling facility and of aluminium hydroxide supplier.

Stability and stability dating period

Stability of Phase 1 and Phase 2 Material

Initial studies by WRAIR on Phase 2 material, limited to potency testing only, showed the vaccine to be intrinsically stable over a very long period of time. Phase 1 and Phase 2 clinical lots filled into stopped vials were stable during storage at 2-8°C for a period of up to 71 months (Phase 1) and 55 months (Phase 2).

Stability Testing of Drug Product manufactured according to Phase 3 Clinical Trial Material manufacturing process

- Currently, the three stability lots of Final Bulk Vaccine remain stable for up to 18 months when stored at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$
- Currently, the three stability lots of Final Vaccine remain stable for up to 18 months when stored at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$.

Stability data will be generated on the first three consistency lots manufactured according to the commercial process.

Currently the product has received a shelf life of 24 months in Australia; 18 months in USA and 12 months in EU.

Viral and microbiological safety

The cell line used for the manufacturing of IC51 (JE-PIV) is a WHO Vero cell WCB established at the Intercell Biomedical Ltd facility and deriving from WHO Vero MCB manufactured by BioReliance, US. The WCB has been manufactured according to cGMP. Appropriate microbiological and virological testing has been performed on the Vero Cell Bank. A TSE risk assessment was performed by an independent TSE expert who concluded that the controls and specifications for starting materials and raw materials of biological origin were adequate.

The Working Virus Seed Bank used for the Intercell process was established at Intercell Biomedical Ltd facility from the master virus seed produced at WRAIR. Appropriate microbiological and virological testing has been performed on the Virus Seed Bank. A TSE risk assessment was performed by an independent TSE expert who concluded that the controls and specifications were adequate

All media are sterile and prepared according to current cGMP guidelines and in compliance with all safety regulations (including TSE). All raw materials of animal origin undergo appropriate testing to ensure absence of potential viral contamination.

All raw materials and reagents of ruminant origin comply with the TSE Note for Guidance EMEA 410/01, current revision. The fetal bovine serum used in IC51 (JE-PIV) manufacture is from Australian source and is covered by a TSE Certificate of Suitability.

Appropriate controls are performed during the process to ensure absence of viral contamination (in the negative control cells, in the viral harvest and in the Final Vaccine Lot) and of microbial contamination (in viral harvests, in protamine sulphate treated material, in the sucrose gradient purified material, in the Drug Substance (pre- and post sterile filtration), in the excipients, in the Final Bulk Vaccine and on the Final Vaccine Lot).

The manufacturing process includes a validated viral inactivation step (formaldehyde treatment) and a validated sterile filtration step for Drug Substance prior to formulation with aluminium hydroxide .

Non-clinical Studies

Pharmacology

Dose related immunogenicity of the Drug Product produced so far was checked in a non-clinical setting, using mice. Potency of the vaccine is determined as an ID50 (Immunizing Dose 50), i.e. the amount(ng)of vaccine needed to be administered to the mice to reach a specified level of antigen neutralisation (50%) in the PRNT assay (by the antibodies produced by the mice), in other words, to reach a certain level of antibody production in mice and of neutralising activity.

The following table summarizes the potency of the DP that has been used in the clinics till date.

Table 1: Antigenic potency of different batches of IC51 and JE-VAX[®] vaccines in mice

Clinical Study	Sample	Manufactured by	Lot No.	ID50 ng (95% CI)
Phase 1	IC51	WRAIR, U.S.A	0574	4 ng (2-7)
	JE-VAX [®]	BIKEN, Japan		15 ng
Phase 2	IC51	WRAIR, U.S.A	0737	2.4 ng
	JE-VAX [®]	BIKEN, Japan	EJN 163A	55 ng (26.0–116)
Phase 3	IC51	Intercell, U.K.	ICB05-501	11 ng (6.7-50)
	IC51	Intercell, U.K.	ICB05-502	8 ng (NC)
	IC51	Intercell, U.K.	ICB05-503	>50 ng ^(*)
	JE-VAX [®]	BIKEN, Japan		NC

Notes: N Ab: Neutralizing Antibody C: Confidence Interval NC: Not calculated

(*) The potency result was inconclusive since it was out of the assay range (i.e. dilutions range of mouse inocula set to determine the standard curve). Retested after 6 months storage at 5°C ± 3 °C, potency result of 38 ng was obtained.

In addition to immunogenicity, protective efficacy of the vaccine was ascertained by immunizing mice with different amounts of vaccine preparation followed by a lethal dose (800 times the dose required to kill 50 % of mice) of a pathogenic JEV strain, SA-14. It must be noted that this experiment was done from the material produced for Phase 1 trial. The results are summarized in table 2.

Table 2: IC51 and JE-VAX® vaccine protective efficacy in mice.

Vaccine	Dose (ng)	Percent Protection	Protection ED ₅₀ (95% CI)
IC51	1500	100 (10/10)	
Lot 0475	150	100 (10/10)	2.6 ng
(Bulk vaccine)	15	80 (8/10)	(0.02 - 7.6)
	1.5	40 (4/10)	
JE-VAX®	600	100 (10/10)	
	60	100 (10/10)	1.5 ng
	6	90 (9/10)	(0.6 - 3.7)
	0.6	20 (2/10)	
IC51	50	60 (12/20)	
Lot 0574	5	60 (12/20)	8.4 ng
(final vaccine)	0.5	15 (3/20)	(2.2 – 86.0)
	0.05	15 (3/20)	

A passive transfer study was undertaken to correlate the PRNT titers that are measured in the laboratory against the survival of mice. It is expected that the PRNT titers are good estimate of the vaccine efficacy.

Non-clinical and toxicology studies

Safety study in animals

All lots (0574, 0737, ICB05-501, ICB05-502 and ICB05-503) used in the clinical trials have passed a general safety test done in Guinea pigs and mice. It was done in the purview of the QA/QC according to the 21 CFR 610.11. The animals were observed for a total period of 7 days. Overt signs of ill health, death or weight loss determined toxicity during the test period. The tests were satisfactory in the result.

Classical toxicology studies (repeat dose) were not performed considering the class of the product (inactivated vaccine containing 6 microgram of antigen, given 28 days apart), the stage of development and clinical experience.

IC51 - Pre and post natal developmental toxicity study in rats

A toxicity study in rats was performed in order to detect effects of vaccinations with IC51 (JE-PIV) on the pre and post natal development. This was done under the conditions of intramuscular vaccine administration before and at the start of pregnancy: the vehicle Control and Vaccine I treatment group received IC51 (JE-PIV) as 3 injections in total given 2 weeks apart (three weeks prior to pairing for mating, a second dose one week prior to mating and a third dose on day 6 of gestation), the Vaccine II treatment group

received IC51 (JE-PIV) as 2 injections in total given 2 weeks apart (one week prior to mating and a second dose on day 6 of gestation). The IC51 (JE-PIV)-specific antibody response was analyzed at different timepoints upon vaccination by PRNT assay. The results indicate that the vaccine had a good profile and no major concern was raised regarding the results of this study.

Clinical

Various clinical studies have been performed during the development of IC51. Phase 1 and Phase 2 trials were completed by WRAIR, and revealed a promising safety and immunogenicity profile of the candidate vaccine.

Phase 1

Country: USA. 28 enrolled subjects in 4 groups. The majority of study volunteers immunized with 0.4 mcg of vaccine showed poor immunogenicity. There was marginal immunogenicity after vaccination with 2 or 3 doses of 2.0 mcg of vaccine.

Phase 2 (Lyons et al., 2007)

Country: USA. The aim of the study was to evaluate the immunogenicity of two different dosages of IC51 given in two-dose or three-dose schedules and to compare this with the licensed JE-VAX® vaccine. There were 100 enrolled subjects in 4 groups. This study showed that IC51 is safe and induced good immune responses to IC51. At day 56, 95-100% of subjects in the IC51 groups had seroconverted (defined as JE PRNT >10), compared with 74% recipients of the currently licensed JE-VAX®. A vaccine dose and schedule was identified for Phase 3 evaluation (6 mcg IM in two doses, 28 days apart). IC51 exhibits a safety, reactogenicity and immunogenicity profile that appears to be superior to the licensed JE-VAX®.

Phase 3

The clinical Phase 3 study program performed by Intercell AG encompasses the following clinical studies which are summarized below.

Study IC51-301 – pivotal immunogenicity trial (Tauber et al, 2007)

An observer blinded, controlled, randomized Phase 3 study in 867 healthy adult subjects in USA and Europe. In this study, the immunogenicity of the IC51 vaccine was compared to that of an active comparator (USA licensed) JEV vaccine, JE-VAX®. IC51 was found to be non-inferior to JE-VAX® with respect to both co-primary endpoints, seroconversion rate (SCR) and geometric mean titer (GMT) at day 56. Both IC51 and JE-VAX® had an acceptable safety profile. Local tolerability findings suggest a more favorable profile for IC51 as documented in the subject diaries, especially for itching, swelling, hardening and redness at the injection site.

Study IC51-302 – pivotal safety trial (Tauber et al, 2008)

A double-blind, placebo-controlled Phase 3 study, conducted in USA, Europe and Australia (incl. New Zealand), with a total of 2,012 randomized healthy adult subjects treated with IC51, and 663 randomized subjects treated with placebo (aluminum

hydroxide adjuvant in saline). The primary objective of the study was to investigate the safety and the tolerability of the IC51 vaccine through 4 weeks after the last vaccination. Safety, as well as local and systemic tolerability, was favorable, with adverse event rates comparable to the placebo treatment.

Study IC51-303 – long term immunogenicity and safety follow-up trial (ongoing) (Schuller et al, 2008)

An uncontrolled, multi-center, Phase 3 follow-up study. In 3258 subjects from 44 sites having completed studies IC51-301 or IC51-302 and having received at least one vaccination, solicited side effects were assessed for the long term safety of IC51 through 6 months after the first vaccination. In addition, 180 subjects will be followed up for immunogenicity for at least five years. Of these 180 subjects there is already data available on safety and immunogenicity 24 months after the first vaccination.

The seroconversion rate at 24 months after the first IC51 vaccination was 81.8%. The respective GMT was 44.3 at Month 24. IC51 was well tolerated from 2 to 24 months after first vaccination. Most AEs were mild or moderate in intensity and none was considered to be treatment related.

Study IC51-304 – rapid immunization regime (ongoing) (Schuller et al, 2009)

An observer-blinded, controlled, randomized Phase 3 study in 375 healthy adult subjects in Germany and Northern Ireland. The aim of the study is comparison of a rapid immunization scheme comprised of one dose of either 12 mcg or 6 mcg of IC51 vaccine versus the standard 2 x 6 mcg dose vaccination schedule.

The 2 x 6 mcg dose schedule of IC51 led to higher GMT and SCR as the 1x12 mcg dose regimen. This study showed that the IC51 2 x 6 mcg regimen was the most appropriate and effective dose and yielded protective titers in 97.3% of subjects 7 days after the second dose of IC51..

Study IC51-305 – long term persistence and booster follow-up trial

An open-label, non-randomized Phase 3 study in 349 healthy adult subjects having completed study IC51-304 to analyze the long term persistence of immunogenicity of three schedules of IC51 for up to 2 years after the primary vaccination. 24 months after the primary immunization, 48.3% of subjects who completed the full primary immunization (2x6 mcg) showed persisting protective antibody titers. Booster doses were administered to subjects with negative immune status at 11 or 23 Months after the primary immunization. Regardless of treatment group or timing, all booster doses led to SCR of $\geq 99\%$.

Study IC51-308 – concomitant vaccination trial

A randomized, controlled, single-blind Phase 3 study in Austria and Germany to investigate the effect of concomitant vaccination of IC51 with HAVRIX®1440 in 192 healthy adult subjects. IC51 + HAVRIX®1440 was found to be non-inferior to IC51 + placebo and HAVRIX® + placebo with evaluation of GMT for anti-JEV neutralizing antibody at Day 56 and HAV antibody at Day 28. IC51 + HAVRIX® had a favorable safety profile, with adverse event rates comparable with the other treatment groups, and a favorable local and systemic tolerability profile.

Study IC51-309 – lot consistency trial

A randomized, controlled, double-blind Phase 3 study in Austria and Germany to compare the human immunogenicity of three manufacturing lots of IC51 in 639 healthy adult subjects. The equivalence between batches could not be postulated as only the comparison of batches A and D resulted in a 95% Confidence Interval for the GMT ratio at Day 56 within the specified acceptance range. The three batches had a similar tolerability profile.

Study IC51-310 – commercial lot consistency trial

A randomized, controlled, double-blind Phase 3 study in Austria and Germany to compare the human immunogenicity of three commercial manufacturing lots of IC51 in 389 healthy adult subjects. The equivalence between batches in terms of SCR and GMT was demonstrated. The three batches had a similar tolerability profile.

Study IC51-311 – booster dose trial

This is an uncontrolled, open-label phase 3 study in healthy adult subjects after primary immunization with IC51 in study IC51-309. The primary objective is to assess the effect of a booster vaccination on immunogenicity of IC51 in terms of seroconversion rate (SCR) at Month 12 after the booster vaccination (Month 27 after primary immunization).

Study IC51-314 – End-of-shelf-life trial

This is an open-label, multicenter, phase 3 study assessing immunogenicity at various time points throughout the shelf-life of a commercial batch of IC51. Three sequential cohorts, each containing 100 subjects, will be enrolled into the study at approximately 12 (cohort 1), 18 and 24 months after filling of the commercial batch of IC51. The primary objective of the study is to assess immunogenicity at Day 56 after the first vaccination.

Study IC51-221 – Pediatric phase 2 trial

This was a prospective, single centre, randomized, active control, and open label, phase 2 optimal dose identification study of immunogenicity and safety of IC51 in 60 healthy Indian children aged ≥ 1 to < 3 years. This study was sponsored by a cooperation partner of Intercell AG, Biological E Ltd. IC51 was equally immunogenic as the licensed comparator JE vaccine and had a favorable safety profile, both in the full (6 mcg) and half (3 mcg) adult dose of IC51. In conclusion, the 3 mcg dose will be used for the development in the pediatric population below 3 years of age.

Further clinical studies are planned to fulfill post marketing commitments as well as to study IC51 in the pediatric population.

Regulatory

Intercell had continuous discussions with different regulatory agencies and national health authorities. They include US FDA, EMEA, PEI from Germany, MPA from Sweden, MHRA from U.K.

Intercell submitted license applications in Australia, US, Europe (through EMEA centralized procedure), Canada and Switzerland. The product has been approved so far in Australia (ATRG No. AUST R 150602); USA (License No. 1790) and EU (Community register no. EU/1/08/501/001 and 002). Decisions from Canada and Switzerland awaited. The product is licensed as “IXIARO” in US and EU while it is licensed as “JESPECT” in Australia.

References

- Eckels et al (1988)** Japanese encephalitis virus live-attenuated vaccine, Chinese strain SA14-14-2; adaptation to primary canine kidney cell cultures and preparation of a vaccine for human use. *Vaccine*. 6(6):513-8
- Srivastava AK, Putnak JR, Lee SH, Hong SP, Moon SB, Barvir DA, Zhao B, Olson RA, Kim SO, Yoo WD, Towle AC, Vaughn DW, Innis BL, Eckels KH (2001).** A purified inactivated Japanese encephalitis virus vaccine made in Vero cells. *Vaccine*. 19(31):4557-65
- Lyons A, Kanesa-athan N, Kuschner RA, Eckels KH, Putnak R, Sun W, Burge R, Towle C, Wilson P, Tauber E, Vaughn DW (2007).** A Phase 2 study of a purified, inactivated virus vaccine to prevent Japanese encephalitis. *Vaccine*. 25(17):3445-53
- Tauber E, Kollaritsch H, Korinek M, Rendi-Wagner P, Jilma B, Firbas C, Schranz S, Jong E, Klingler A, Dewasthaly S, Klade CS.** Safety and immunogenicity of a Vero-cell-derived, inactivated Japanese encephalitis vaccine: a non-inferiority, phase III, randomised controlled trial. *Lancet*. 2007 Dec 1;370(9602):1847-53.
- Tauber E, Kollaritsch H, von Sonnenburg F, Lademann M, Jilma B, Firbas C, Jelinek T, Beckett C, Knobloch J, McBride WJ, Schuller E, Kaltenböck A, Sun W, Lyons A.** Randomized, double-blind, placebo-controlled phase 3 trial of the safety and tolerability of IC51, an inactivated Japanese encephalitis vaccine. *J Infect Dis*. 2008 Aug 15;198(4):493-9.
- Schuller E, Jilma B, Voicu V, Golor G, Kollaritsch H, Kaltenböck A, Klade C, Tauber E.** Long-term immunogenicity of the new Vero cell-derived, inactivated Japanese encephalitis virus vaccine IC51 Six and 12 month results of a multicenter follow-up phase 3 study. *Vaccine*. 2008 Aug 12;26(34):4382-6.
- Schuller E, Klade CS, Wöfl G, Kaltenböck A, Dewasthaly S, Tauber E.** Comparison of a single, high-dose vaccination regimen to the standard regimen for the

investigational Japanese encephalitis vaccine, IC51: A randomized, observer-blind, controlled Phase 3 study. Vaccine. 2009 Mar 26;27(15):2188-93.